ASSESSMENT AND PREDICTION OF ELECTROSHOCK-INDUCED INJURY IN NORTH AMERICAN FISHES

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ABSTRACT

Electrofishing has served as an efficient method for scientific sampling of freshwater fishes since the mid-1900s, but it has become apparent since the 1990's that electroshock can cause fish injury. Electroshock-induced fish injury (damage to hard or soft tissues), which is primarily manifested as vertebral fracture or hemorrhage (broken blood vessels) along the backbone, can be a critical determinant of fish survival. The ability to predict factors influencing fish injury rate (the proportion of injured fish in a sample) would be very useful to biologists. To test the null hypothesis of no effect of electrical waveform (W), voltage gradient (E), and fish size (S) on injury rate, I conducted controlled electroshock experiments on chinook salmon *Oncorhynchus tshawytscha*, rainbow trout *O. mykiss*, channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and hybrid striped bass *Morone saxatalis* x *M. chrysops*. Data collected included electrical stimulus, fish behavioral response (R), length (L) and weight (W), and injury status (present/absent). Vertebral injury was determined using radiography, and hemorrhage by bilateral filleting. My model selection criteria, which was based on Akaike's Information Criterion (AIC), indicated that risks for both types of injury in chinook salmon and channel catfish were best represented by the (W, E, S) model, the (W, S) model for both types of injury in rainbow trout, the (W, E) model for hemorrhage and the (W, E, S) model for vertebral injury in largemouth bass, the (W) model for both injury types in hybrid striped bass, and, that risk for injury in bluegill injury was best described by the null model (no effect of W, E, S). A mechanistic model relating electrical stimulus, the force of contraction, and the resistance to contraction to electroshock-induced injury, using (R) as a surrogate for electrical stimulus, (L) as a surrogate for force of contraction, and vertebral count (V) as a surrogate of resistance to injury, was explored. Application of the mechanistic model (R, L, V) to the pooled data set demonstrated a strong predictive relationship. This model offers guidance for the reduction and prevention of electroshock-induced injury for all species in all situations.

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INTRODUCTION

Electroshock-induced fish injury (mechanical damage to hard and soft tissues) is primarily manifested as vertebral injury or hemorrhage (discharge from broken blood vessels) along the vertebral column (Hauck 1949, Spencer 1967, Sternin et al. 1976, Madden and Houston 1976, Sharber and Carothers 1988a). Over the last decade substantial effort and investigative resources have been directed toward understanding the problem of electrofishing injury (McMichael 1993, Hollender and Carline 1994, Sharber et al. 1994, Dalbey et al. 1996, Thompson et al. 1997, Ainslie et al. 1998, McMichael et al. 1998). Considerable progress has been made, but the various methods, fish species, equipment, and environmental conditions in electrofishing-injury studies conducted, thus far, make direct comparisons of injury rates from the studies problematic. The existing data set, however, can be used to identify potential predictors of fish injury. Technical variables, such as current type (alternating current, AC; direct current, DC), pulsed DC (PDC) pulse shapes and frequencies, evaluated in previous works have potential for predicting fish injury. Prior works identify fish size as a possible predictor of fish injury (Hollender and Carline 1994; Thompson et al. 1997; Ainslie et al. 1998), though the variable has not been directly addressed experimentally.

The great variation in fish injury has led to an interest in identifying accurate predictors of injury. The ability to predict which factors have the greatest influence on fish injury would be a very useful tool for biologists, whether applied to healthy populations or rare and threatened fish. Prognostic models are powerful tools frequently used in studies of clinical outcomes, which have also been used to evaluate risk factors associated with electroshock-induced mortality (Holliman et al. in press a). Critical application of modeling techniques is needed to ensure that these models fit the dataset at hand, without overfitting, and are accurate predictors of outcome (Harrell et al. 1996).

The objectives of this study were 1) to document the scope of the electrofishing-induced injury problem through evaluation of various North American freshwater fishes; 2) to evaluate various models describing the relationship of voltage gradient, DC pulse frequency, fish size, and induced behavioral response to fish injury; and 3) to develop a general model of injury that may be used by biologist to prevent or reduce the occurrence of fish injury. My goal is the development and refinement of prognostic models that identify risk factors for electroshock-induced injury for North American fishes based on a mechanistic foundation. To achieve the goal and objectives, I used data from controlled experiments to test null hypotheses of “no effect” of postulated risk factors on occurrence of injury in electroshocked fishes of various species and sizes.

REVIEW OF LITERATURE

Examination of the existing literature also reveals areas lacking thorough investigation, areas that are fundamental to the use of electricity for capturing fish. For instance, susceptibility to injury may vary among species (Spencer 1967; Thompson et al. 1997), but reports are scarce. Most published studies of electroshock-induced injury have involved salmonids, especially rainbow trout *Oncorhyncus mykiss*. Little is known of injury susceptibility in other fishes. Further, electrofishing is based on evoking responses from fish (e.g., galvanotaxis, psuedo-forced swimming, narcosis, and tetany) that lead to capture. Whether the behavioral responses required for successful electrofishing can be induced without causing fish injury has been questioned, but not evaluated. Furthermore, the role of voltage gradient (i.e., electric field intensity) in fish injury is unclear. More work must be done to delineate the predictive value of these variables in terms of fish injury. Conceptual models for fish injury can be gleaned from prior works on electroshock-induced injury, electrofishing theory, and electrobiology.

Voltage gradient (E) is a potential risk factor for electroshock-induced injury (Hudy 1985; McMichael 1993). Haskell and Adelman (1955) noted that in isolated fish muscle, no muscular contraction occurs below a specific voltage threshold. As voltage increases above the threshold for contraction, muscle contraction increases in a step-wise manner until complete contraction occurs. Upon reaching complete contraction, further increases in voltage have no effect.

Increasing voltage results in longer contraction times prior to relaxation, the onset of narcosis (Biwas and Karmarkar 1979). Reynolds and Kolz (1988) maintained that the dependence of injury rate on voltage gradient has been demonstrated, at least in part, by several controlled studies. Sharber et al. (1994), citing Reynolds and Kolz (1988) and Cowx and Lamarque (1990), reported that the current paradigm of fish injury is that fish injury results from severe muscular contractions associated with tetany. Tetany is associated with the most intense area of the electric field (near the anode), the area having the largest voltage gradients. Therefore, the paradigm assumes that the principal cause of electrofishing injury is high voltage gradient. This notion has led to the assumption that vertebral injury can be avoided by operating electrofishing equipment at a level that elicits electrotaxis and narcosis but avoids tetany. Sharber et al. (1994) challenged the current paradigm with data on rainbow trout indicating that injury can occur at voltage gradients below that required for narcosis, and therefore below that associated with the onset of tetany. Sharber and Black (1999) went on to propose that fish injury during electrofishing is a result of epilepsy; however, they provided no proof for their hypothesis. To date, the debate over the relation of voltage gradient to fish injury has not been settled with experimental data published in the scientific literature.

Electrical waveform, a factor that includes current type, DC pulse rate, and DC pulse shape is identified as a potential risk factor for electroshock-induced injury. Alternating current (AC), direct current (DC) and pulsed DC (PDC) have been used in North America to capture fish since the mid-1900s (Rayner 1949; McLain and Nielsen 1953; Pratt 1954). Early electrofishers were aware that the type of electrical current used could influence the likelihood of fish injury. That AC could cause severe fish injury (e.g., fractured vertebrae, ruptured haemal artery, hemorrhaging) was confirmed in large rainbow trout during a rescue attempt (Hauck 1949). On the other hand, use of DC resulted in no injury of trout or salmon parr during an evaluation of a DC electrofishing apparatus (Smith and Elson 1950). Contrary to the description of interrupted DC having benign narcotic-like effects on fish (Rayner 1949), severe internal injuries in brook trout *Salvelinus fontinalis* were reported in an evaluation of fish guidance using 1-5 Hz PDC (McLain and Nielsen 1953). Bary (1956) reported that repeated shocking of golden grey mullet *Mugil auratus*, bass *Marone labrax*, and flounder *Platichthys flesus* with AC, DC, and PDC failed to produce detectable internal hemorrhages or injuries to muscles or bones during an evaluation of the effect of electric fields on marine fishes. Conversely, in a report evaluating mark-recapture using PDC electrofishing, Klein (1967) reported that spinal deformities were common injuries in trout subjected to electrotaxis.

Haskell and Adelman (1955) refer to the summation of inadequate stimuli effect to explain the action of pulsed DC on fish, where the muscular contractions under PDC at 20-Hz increased above those obtained with a single pulse of the same voltage. The increased efficiency of PDC is accomplished by violent muscle contractions. The physiological effects of DC and PDC are fundamentally different. Galvanonarcosis, induced by DC through central nervous system depression, has been likened to a chemical narcosis accompanied by muscle relaxation, whereas PDC induces immobilization though stimulation of the central nervous system and muscle tetany or contraction (Halsband 1967). The action of PDC on the musculature has been described as cramp leading to constant irritation (Halsband 1959).

Today, PDC is used extensively for electrofishing. Sharber and Carothers (1988a) demonstrated that DC pulse shape influenced the likelihood of injury of large rainbow trout. Sharber et al. (1994) demonstrated a curvilinear relation between electrofishing injury and DC pulse frequency for rainbow trout, with injury occurring more frequently with higher frequencies (i.e., 60-Hz and higher) than lower frequencies. This relation between pulse rate and injury has been confirmed repeatedly in other studies (McMichael 1993; Dalbey et al. 1996; Ainslie et al. 1998). The likelihood of tetany also increases with frequency, lending credence to the notion of tetany-induced injury.

Fish size may be a predictor of electroshock-induced injury. The biology of an organism is affected by its size at every organizational level (Goolish 1991). Thus, dissimilar injury rates between larger and smaller fish may be expected, a notion supported by several studies (Taylor et al. 1957, Hollender and Carline 1994, Dalbey et al. 1996, Thompson 1997, McMichael 1998, Ainslie et al. 1998). Some features of fish, notably the relations between lengths, areas, and volumes, change drastically with size (Wilkie 1977). Scaling, the structural consequences of a change in size among similarly shaped animals, may provide insight into the mechanism of electrofishing injury. Length is a good measure of similarly-shaped animals (Schmidt-Nelson 1977). Fish length has been linked to injury in previous studies (Taylor et al. 1957, Hollender and Carline 1994, Dalby et al. 1996, Thompson 1997, McMichael et al. 1998, Ainslie et al. 1998). When making comparisons of size of animals of different shape, mass has the advantage that almost all animals have a density close to 1.0, making mass a good measure of total volume, a good simplification (Schmidt-Nelson 1977). Whether fish mass (weight) is a predictor of injury has not been explored previously.

The usefulness of fish reaction to electroshock as a predictor of injury has also not been evaluated previously. Fish swim by alternately contracting the left and right lateral musculature to create cyclic oscillations (Wardle 1977). Fish behaviors (taxis, narcosis, psuedo-forced swimming, tetany) in electrified water indicate an interruption of normal neuro-motor functioning regardless of whether the interruption is caused by effects on the central nervous system, a stimulus-response type reaction, or by the direct action of electric current on nerves and muscle. Lamarque (1967) held that stimulation of muscles on both sides of the body simultaneously is the mechanism leading to fracture or dislocation of vertebrae in fish exposed to PDC. Reynolds and Kolz (1988) hypothesized that pulsed waveforms are damaging to fish when applied at power levels exceeding those for narcosis, a state of muscle relaxation. Application of excessive power densities causes tetany (muscle contraction) simultaneously on both sides of the fish, thus compacting the vertebral column and its associated blood vessels and nerves to the point of physical damage. Sharber and Carothers (1988b) argued that a power level high enough to induce narcosis in large trout can, and does, induce vertebral injury in these fish. They refuted the hypothesis of Reynolds and Kolz (1988) that the electrotaxis-narcosis reaction can be elicited without causing the muscular seizures that cause compression fractures of vertebral column. Sharber and Black (1999) provided induced-epilepsy as the origin of electrofishing injury. Myoclonic jerks (i.e., the simultaneous contraction of parallel myotomes that frequently accompany the onset of epileptic events) during seizures associated with electrotaxis and narcosis were credited as the source of injury. Although the origin of the muscle reaction (stimulus-response versus direct action versus epilepsy) and thresholds for injury (electrotaxis, narcosis versus tetany) are unclear, there is agreement that fish injury results from simultaneous muscle contraction on both sides of the fish body.

The morphological variation among species may provide insight into size- and species-related susceptibility to electroshock-induced injury. The total force generated by muscles is proportional to the surface area of the cross-section perpendicular to the fiber direction and the total power generated is proportional to the muscle volume (Videler 1993). The backbone is the primary site for elctroshock-induced mechanical injury. The morphology and biomechanics of the backbone varies considerably among fishes. That simultaneous bilateral muscle contraction may cause fish injury is accepted. It follows that fish size (using length and weight as a surrogate), may indicate susceptibility to injury and vertebral count may be a useful index of resistance to injury.

Lamarque (1990) reported that fish injury results from "violent contractions produced simultaneously by the current of both sides of the fish body following direct excitation and hyper-reflexivity". The nervous system in fish consists of the brain and spinal cord (the central nervous system (CNS), motor and sensory nerves linking effector organs and receptor cells with the CNS, and the autonomic system controlling the visceral functioning (Bone et al. 1995). The mechanism of fish behavior to waterborne electric fields has been explained by stimulus-response, direct action of local electricity on local nerves and muscles, and epilepsy. Fish behaviors (taxis, narcosis, psuedo-forced swimming, immobilization) in electrified water indicate an interruption of normal neuro-motor functioning.

Lamarque (1990) clarified fish electrophysiology by explaining the action of DC and PDC on the neuro-muscular system in fish. Direct current acts on the body cells (electrotonus) and muscle, but not on nerve fibers. Pulsed DC acts on nervous and muscle fibers in accordance with Pflügger's Laws. Under the action of DC, the laws of electrotonus apply. Accordingly, the body cell is either facilitated (increase in excitability) or inhibited (a reduction in excitability). When a body cell is stimulated it transmits its stimulation to the fiber, thereby producing either an increase in the reflex pulses from a higher path, or a direct stimulation of the fiber, depending on the voltage. If inhibition occurs, stimulation from the higher path is reduced. The size selectivity demonstrated by electrofishing is hypothesized to result from increased body voltage and longer nerves. Whether a nerve cell is excited or inhibited depends on its orientation to the anode and cathode. The degree of excitation is dependent upon the potential difference between the extremes of the nerve cell. The angle and the length of the nerve affects the potential difference intercepted (Lamarque 1963). However, Rushton (1927) found that the longer the interpolar length the more excitable the nerve up to a point, which in the frog sciatic-gastrocnemius preparation was 20 mm. There was a marked decrease in excitability beyond this point. Basically, there was an exponential decrease in the threshold of excitability to length, but at lengths beyond 40 mm there was a constant threshold (Rushton 1927). The direct action of voltage on nerves longer than 40 mm cannot explain the increasing susceptibility to electricity in larger fish.

There is a striking similarity between electrofishing and some toxicology studies regarding vertebral column injuries and hemorrhaging. Bengstton (1975) reported on hemorrhage observed in fish resulting from vertebral collapse, which caused damage to the surrounding tissues and blood vessels. Acute muscle contraction was hypothesized as the mechanism leading to vertebral damage, with parasitic infection, electrical current, and toxic substances as possible causative agents (McCann and Jasper 1972, Bengsston 1974, Baumann and Hamilton 1984). Strong localization of vertebral damage in the posterior portion of the axial skeleton was deemed indicative of acute muscular contraction (Spencer 1967, McCann and Jasper 1972). The localization of fractures in the caudal region resulted from the vertebral column being surrounded by the greatest muscle mass at the base of the caudal area. The abdominal-cuadal junction is the most probable site for overloading the vertebrae when there are occasional muscular convulsions resulting from affects on the neuro-muscular complex (Bengtsson 1975).

The axial skeleton in teleost fishes is analogous to a long column composed of short, separate elements, the centra (Laerm 1976). The vertebral centra in fishes are amphicelous, resembling biconid hour-glass shaped cylinders with concave ends. The biconid is formed in compact bone, while longitudinal bars of spongy or cancellous bone span the length of the lateral surface of the centrum. Different types of processes extend from the centra, depending on vertebra location along the vertebral column. All vertebrae possess a neural arch and spine extending dorsally from the centrum. The abdominal vertebrae possess aphophyses (i.e., lateral projections) that connect with ribs enclosing the abdominal cavity, vertebrae in the caudal region have a haemal arch and spine on the ventral side, but lack ribs. The intervertebral articulations are amphiarthotic (i.e., a type of articulation where the bony surfaces are connected by cartilage, Thomas 1981). The vertebrae are separated by thin, notochordially derived, fibrocartilage rings. Inter-vertebral ligaments provide stability to the joint. Considerable manual force is required to dislocate fish vertebrae. The intervertebral ligaments are tough, inelastic, collagen fibers which help to prevent dislocation, limit the degree of lateral bending, and keep the internal intervertebral ring-like ligament of each joint in position between the vertebral margins (Symmons 1979). Dorsal and ventral longitudinal ligaments, which are threaded through the neural and haemal arches of the vertebrae, span the length of the vertebral column and contribute to the stability of the structure (Laerm 1976, Symmons 1979, Videler 1993).

Weihs (1989) described the basic mode of aquatic locomotion as a wiggling motion produced by the lateral musculature sending waves of sideways displacement of the vertebral column in the caudal direction. Undulation (anguilliform swimming) occurs when all vertebrae of the fish have flexible joints. Herring and other clupeiform, elopiform and salmoniforme fish are anguilliform swimmers. In these lower teleosts lateral undulations are made easier by the neural arches and lateral ribs being moveably attached and able to slightly rotate on their centra. The pleural neural spines are not joined distally. The ribs do not impede lateral movement being attached to very short parapophyses. In the caudal region, the neural and haemal spines are firmly attached to the centra, thus the tail is strengthened and stiffened (Symmons 1979). In comparison, higher teleosts have comparatively stiff pleural regions. In these fishes, the anterior vertebrae are linked together by apophyses and well-developed connective tissue. Lateral movement is very limited in the pleural region, which remains straight while the tail is flexible. Other adaptations include neural arches that are almost immovably attached to their centra, and ribs that attach to strong broad parapohyses which support the swim bladder beneath. The vertebral joints are without much movement because of apophyses and strong ligaments limit movement in the pleural region, preventing the parapophyses from overlapping and disturbing the contours of the swim bladder. In the caudal region the vertebrae may be much smaller but all the joints to the last are flexible so that the caudal fin region is very mobile (Symmons 1979).

Strong physical (muscular) reactions may be responsible for electrofishing injury (Reynolds and Kolz 1988, Sharber and Carothers 1988b, Lamarque 1990, Sharber et al. 1994, Sharber and Black 1999). Tetanic muscular contractions, muscular convulsions, and apparent seizure have caused vertebral fracture and hemorrhaging in fish exposed to toxins (McCann and Jasper 1972, Bengtsson 1974, Bengtsson 1975, Holcombe et al. 1976, Baumann and Hamilton 1984). Larger fish, based on within species comparisons, are more susceptible to injury. There is a strong correlation between fish size (mass and length) with age. Changes in fish vertebrae structural integrity accompanying maturation (i.e., decrease in strength) coupled with increased muscle mass and power may account for the increased likelihood of injury in larger fish. This is because the power required increases approximately with the cubed root of the swimming speed (Webb 1975). The total force generated by muscles is proportional to the surface area of the cross-section perpendicular to the fiber direction. Further, the total power generated is proportional to the muscle volume (Videler 1993).

Because the vertebral column is the key support structure for the muscle attachments used in swimming, the structural integrity of the vertebral column is a critical determinant of teleost fish survival (Gray 1957, Hamilton et al. 1981). Bone is a specialized type of connective tissue consisting of an organic matrix, minerals, and water. The organic matrix is about 90% collagen, a protein, with the remaining percentage being mucopolysaccarides, mucoproteins, and lipids. Calcification and mineralization of the vertebrae occurs, within and around the collagen fibers, during bone development and maturation. The mineral content of fish vertebrae is primarily calcium, phosphate, and hydroxide crystals (hydroxyapatite $[Ca_{10}(OH)_2(PO_4)_6]$ (McElhaney 1966, Nusgens et al. 1972). The structural integrity of bone may be described by mechanical properties such as strength, elasticity, and energy-absorbing characteristics (McElhaney 1966, Hamilton et al. 1981).

The mechanical properties of vertebrae from many fish species have been evaluated, primarily in a toxicological context (Hamilton et al. 1981, Bauman and Hamilton 1984, Hamilton and Haines 1989, Hamilton and Reash 1988, Dwyer et al. 1988, Mehrle et al. 1982, Van Den Avyle et al. 1989). Vertebrae mechanical properties are determined by bone quality (composition) and quantity (density). Changes in bone composition and density that may alter vertebral structural integrity and mechanical properties accompany the maturation of young animals into adulthood (Hamilton et al. 1981).

Hamilton et al. (1981) found, in a study evaluating the mechanical properties of brook trout, channel catfish, and bluegill vertebrae that mechanical properties of vertebrae changed with age and differed among the species. In the study, fish length was highly correlated with vertebra size. Because mechanical properties incorporate size, comparisons should only be made among fish of the same size having vertebrae of similar size. Bone density (mg/cm^3) was positively correlated with vertebral strength and elastic limit. Bone strength increased with bone density in channel catfish that were 6 months or older. A decline in bone density observed in older catfish (between 12 and 24 months) appeared to be responsible for a decline in vertebral strength. There was a strong correlation between vertebral toughness and bone density in 2, 9, and 12 month old bluegill (41, 56, and 100 mm, Hamilton et al. 1981). Considerable variation in vertebral density, which may be used as an indicator of vertebral strength, has been reported among fish species. Mehrle et al. (1982) reported that biochemical composition and bone density are important factors in determining vertebral strength. A lack of vitamin C may lead to fragile vertebrae through a decrease in collagen. There is a strong correlation between bone density and vertebral strength. Vertebral strength varies among species and ages of fish. Further, vertebral strength varies along the vertebral column, which is likely associated with muscle function and size.

METHODS

This study was based on two assumptions. The overriding assumption was that the relationships between fish injury rate and the experimental variables that I could evaluate under controlled conditions would be similar to that occurring during electrofishing. Further, I assumed that induced fish behavioral response applies equally to any aquatic setting — laboratory or field. Fish response is the most meaningful basis for comparability of electrical effects between controlled and uncontrolled environments.

Fish response depends on the *in vivo* power achieved in a fish regardless of extraneous factors; see Kolz (1989) for a detailed discussion. For a given level of *in vivo* power, a fish will exhibit the same response even though its surroundings may vary. Thus, a fish's response indicates that a threshold of electrical field intensity has been reached. The threshold-response relationship is useful for inferences about laboratory results as applied to field operations (Holliman et al. in press a; Holliman et al. in press b). Similarly, electroshock-induced injury results from a physical response to *in vivo* electrical stimulus.

Experimental Procedure.—An experiment consisted of a set of treatments (electrical exposures) to individuals of the same species under controlled (tank) conditions. In each experiment, fish from two size groups, designated as large and small, were exposed to combinations of electrical waveform and voltage gradient, or used as controls. Fish were exposed to electrical treatments for a period of 3 s. The electrical waveforms applied in an individual experiment reflected common usage by biologist for capturing the species while electrofishing, except for DC. Direct current was applied in all experiments to provide a basis for comparison. The number of electrical waveforms and voltages evaluated during an experiment were determined by fish availability. Ideally, three electrical waveforms were applied at three voltage levels to two size groups of fish. Twenty fish were assigned to each experimental group, defined by the voltage, waveform, fish size group combinations. Two additional groups (N = 20), one for each fish size group, were used as controls. Thus, the total number of fish in a full experiment was 400. Fish in the control groups were subjected to the same procedures as fish in the treatment groups with the exception of applying electrical power to the tank. The experiments were conducted at aquaculture centers and research stations from June 1998 through October 2000.

The experimental protocol was the selection of one fish at a time from the appropriate holding tank (fish were segregated by size group, large or small); application of a randomly-assigned treatment; classification of behavioral response; collection of fish length (mm) and weight (g) measurements; and evaluation of injury status. Pulsed DC waveforms were square waves of 6 ms duration, pulsed at 15, 30, or 60 Hz, (9%, 18%, or 36% duty cycle) except for the 15-Hz gated burst waveform (15-Hz GTB) which was a complex waveform with four DC pulses of 880 μS separated by 880 μS delivered at the rate of 15-Hz (5%

duty cycle). Direct current output was continuous with a small ripple component (Figure 1).

The voltage levels applied in the experiments were determined immediately prior to each experiment. An iterative process was used to find applied voltage levels of the electrical waveforms to be applied in the experiments that evoked a range of behaviors similar to those observed during electrofishing. Fish used during the preliminary testing were not used in the experiments. Essentially, a fish from each size group was exposed to a voltage level and behavior to electrical treatment was observed. The treated fish was then removed from the tank, the applied voltage varied, and a naïve fish was then exposed to the new treatment. This process was repeated until the three voltage levels were established.

Fish behavioral response during the treatment was evaluated immediately post-treatment, with videotape review for later confirmation and refinement of the initial response classification. Each fish was removed from the test tank immediately after treatment and killed by immersion in an overdose concentration of tricaine methanesulfonate (MS-222), then measured for length and weight. Fish behavioral responses to the electrical treatments were classified using an ordinal scheme based on the system recommended by Sternin et al. (1976) for occasions when a detailed classification of fish response is not needed.

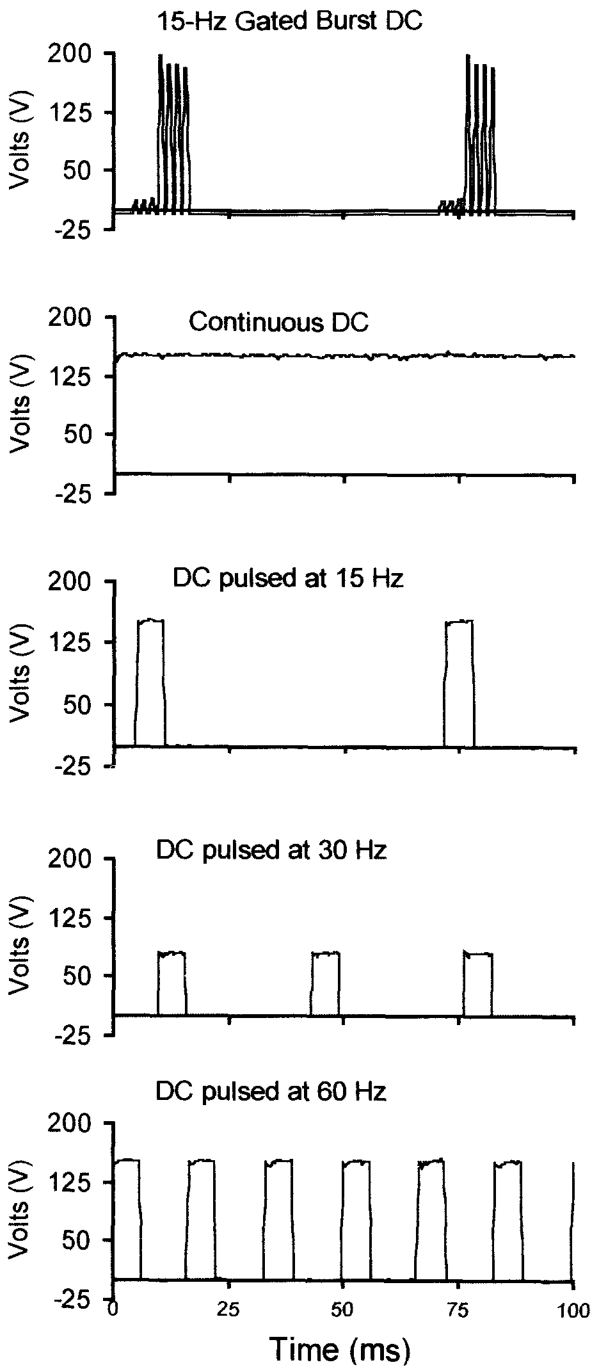


Figure 1.— Electrical waveforms. Examples of the voltage waveforms used in the series of electrofishing-induced injury experiments.

Accordingly, fish response to the treatments was classified as escape, forced swimming, or immobilization, evoked responses of increasing perceived severity. These behaviors are useful for, and observable while, electrofishing. Escape was defined as behaviors from the first tremor at current flow up to rapid, non-oriented (with regard to the electrodes) swimming while maintaining equilibrium. In all cases, fish were shocked with the head directed toward the cathode to aid in the identification of anodic galvanotaxis, if it occurred. Forced swimming included taxis and psuedo-forced swimming. Taxis involved an immediate 180° turn, without touching the sides of the tank, followed by upright swimming to the anode. Dis-equilibrated or unbalanced swimming was described as psuedo-forced swimming. Immobilization was characterized by the complete cessation of swimming motions. The escape response has little value for electrofishing, whereas taxis, psuedo-forced swimming and immobilization increase the likelihood of capture. However, some forms of forced swimming (e.g., rapid, erratic, unbalanced swimming) make capture difficult.

Fish injury was identified via radiography and bilateral filleting. A portable X-ray device was used to radiograph the experimental animals at the study sites. Dorsoanterior and lateral projection radiographs of each fish were made on 24 x 30 cm Kodak MR2000 film using MR2000 intensifying screens, or on 35 x 43 cm regular speed film using regular intensifying screens, depending on fish size. The MR2000 film/screen system has a higher resolution than conventional screen-film radiography (Bushong 1980). Multiple fish from a size group were radiographed simultaneously on individual sheets of film, with the radiographic technique used being dependent upon fish size. Operators of the X-ray machine wore radiation-monitoring devices, a lead apron, and practiced radiation protection (Bushong 1980). After radiography, fillets were removed from each side of the fish to expose the vertebral column. The vertebral column and the lateral musculature (fillets) were then inspected for evidence of injury (broken blood vessels).

Fish injury was rated using the classification system proposed by Reynolds (1996). Accordingly, vertebral injury was categorized as class 0 for no apparent injury; class 1 for compression of vertebrae; class 2 for misalignment of vertebrae; or class 3 for fracture of vertebrae or complete separation of adjacent vertebrae. Hemorrhage status was classified as class 0 for no hemorrhage; class 1 for one or more wounds in the muscle, not associated with the vertebral column; class 2 for one or more small (≤ width of two vertebrae) wounds on the vertebral column; or class 3, one or more large (≥ width of two vertebrae) wounds on the vertebral column. Injury was evaluated without knowledge of the treatment applied to the individual fish.

Equipment.—The tests were conducted in a commercially-manufactured, rectangular 168-cm × 42-cm, fiberglass tank filled with water to a depth of 40 cm. Available hatchery water was continuously supplied to the tank during the experiment using a flow-through system where the water depth was maintained at a constant level by a stand-pipe. Ambient water conductivity (μS/cm) and temperature (C) in the tank were measured each day of an experiment, at the outset and conclusion. Identical steel plates served as the tank electrodes; these were placed parallel to each other 125 cm apart and covered the entire cross-sectional area of the tank. Plastic screens prevented fish from making contact with the electrodes and reduced the effective length of the tank to 118 cm. The power supply for the exposure tank was a Smith-Root Model-15 backpack electrofishing control unit, modified to allow fine adjustment of the output voltage and programmed to ensure each electrical treatment was applied for 3 s. Electrical energy was supplied to the control unit via commercial 110 V AC. A calibrated, digital oscilloscope was used to confirm the potential difference across the electrodes and the waveform applied during treatment of each fish.

The electric field generated in the tank between the electrodes was homogeneous (i.e., a linear change in voltage along the length of the tank) and was described by $y = 1.84 + 0.78x$, where y is the percent of applied voltage and x is the distance (cm) from the anode. The voltage gradient between the electrodes was determined for each electric treatment applied in the individual experiments by multiplying the regression slope (0.78%; 95% CI 0.776-0.783) by the voltage applied. The electric field was homogeneous across water conductivities of 10, 100, and 1000 μS/cm and applied voltages from 50 to 1,050 V (Holliman and Reynolds 2002).

Statistical Analysis.—Descriptive statistics, including means and standard deviations, or counts and percentages or proportions, were reported. The null hypothesis that the mean lengths and weights of the two size groups of fish within an experiment were equal was evaluated with two-sided, two-sample t-tests using the SAS TTEST procedure (SAS 1999). The null hypothesis of equal variances in the samples was evaluated and the appropriate t-test (assumption of equal versus unequal variances) procedure performed.

The injury classifications were converted from the perceived severity index to a binary status (injured versus uninjured) for statistical analysis (Thompson et al. 1997) with hemorrhage and vertebral injury analyzed separately (Schill and Elle 2000). In the event of multiple injury sites (e.g., class 1 and class 2 injuries in the same fish), only the most severe injury was reported. Associations between the experimental variables and injury rate were evaluated in a pair-wise fashion, in marginal tables. The relation of fish behavioral response to electroshock and injury was evaluated in a similar manner. The SAS FREQ (SAS 1999) procedure was used in the analysis. Fisher's two-sided exact test was used to test the null hypothesis of no association between the treatment and injury rate, with a P-value of less than 0.025 being considered statistically significant. The strength of significant associations were estimated using relative risk (RR), the risk of injury for one group compared to another group, $RR = p1/p2$, where p_1 and p_2 were proportions of injured fish within the experimental groups. A 95 percent confidence interval (Efron 1979, Manly 1997) for each relative risk measure was estimated by bootstrapping.

The Kruskal-Wallis test was used as a nonparametric test of the null hypothesis that the distribution of the response variable (hemorrhage rate, vertebral injury rate, and induced behavior) was the same in multiple populations. Induced behavioral response is ordinal in perceived severity, but the distance between the responses cannot be assumed equal. Hence, modified ridit scores were used in the analysis. The Cochran-Armitage trend test, two-sided, was used to evaluate the association of voltage gradient and injury (Stokes et al. 1995).

Species-Specific Model Selection and Inference—The data sets generated by the series of controlled experiments were used to evaluate candidate models relating the experimental variables to fish injury. Fish injury was treated as a dichotomous variable, with injury detected by bilateral filleting and radiography evaluated separately. Predictive models for each species were evaluated. Logistic regression has emerged as the statistical method of choice for predicting dichotomous outcomes, such as injury status (Tu 1996). A set of *a priori* models (Table 1) describing the relationships of fish behavioral response (R), voltage gradient (E), electrical waveform (W), and fish size (S) to electroshock-induced injury, in the form of logistic regression models, were evaluated for each species. The SAS LOGISTIC (SAS 1999) procedure, using

Table 1.—The candidate set of models. The candidate set of models (determined *a priori*) describing the relationship of voltage gradient (E), electrical waveform (W), and fish size (S) to electroshock-induced injury that were evaluated for each fish species.

Model	Injury is related to	Statistical model
Null	random outcome	$\log[p/(1-p)] = \beta_0$
(E)	voltage gradient	$\log[p/(1-p)] = \beta_0 + \beta_1 E$
(W)	waveform	$\log[p/(1-p)] = \beta_0 + \beta_1 W$
(S)	fish size	$\log[p/(1-p)] = \beta_0 + \beta_1 S$
(E, W)	voltage gradient and waveform	$\log[p/(1-p)] = \beta_0 + \beta_1 E + \beta_2 W$
(E, S)	voltage gradient and fish size	$\log[p/(1-p)] = \beta_0 + \beta_1 E + \beta_2 S$
(W, S)	waveform and fish size	$\log[p/(1-p)] = \beta_0 + \beta_1 W + \beta_2 S$
(E, W, S)	voltage gradient, waveform, and fish size	$\log[p/(1-p)] = \beta_0 + \beta_1 E + \beta_2 W + \beta_3 S$

effect parameterization, was employed in the analysis. The Hosmer and Lemeshow goodness-of-fit (GOF) test was used to evaluate the hypothesis that an individual model fit the data well (Hosmer and Lemeshow 2000). Information-theoretic methods (Burnham and Anderson 1998) and the area under the receiver-operating characteristic curve (ROC; Hanley and McNeil 1982; Hosmer and Lemeshow 2000) were used to select a single, best model from the candidate set.

Akaike's information criterion (AIC), a consistent estimator of the Kullback-Leibler discrepancy between the distribution that generated the data and the model approximating it (Buckland et al. 1997), was used to compare models. Smaller AIC values indicate smaller losses of information (Buckland et al. 1997; Thompson et al. 1997; Burnham and Anderson 1998; Franklin et al. 2001). Because AIC is on a relative scale, it is not the actual model AIC value that is important, but the differences in AIC values between models. The models were ranked by Δ_i ($\Delta_i = \mathrm{AIC}_i - \min \mathrm{AIC}$), the difference in the AIC value between the model i and the model with the smallest AIC value. The larger the difference in Δ_i the less plausible the fitted model to be the best. Models with $\Delta_i \leq 2$ should be considered for inference, those with $\Delta_i > 2$ have considerably less support for being the best. Models with $\Delta_i > 10$ fail to explain substantial variation in the data (Burnham and Anderson 1998). Normalized Akaike weights were calculated for each model, using $\omega_i = \exp(-\Delta_i/2) \Big/ \sum_{r=1}^{R} \exp(-\Delta_r/2)$ for comparison of model probability or plausibility (Franklin et al. 2001).

The area under the ROC curve is a plot of model sensitivity (the probability of an injured fish being correctly classified as injured by the model) versus model specificity (the probability of uninjured fish being correctly classified by the model) for an entire range of possible probability cutpoints (i.e., an observation with an estimated probability exceeding the probability cutpoint will be classified as an event, otherwise a nonevent). The area under the ROC curve is a measure of model predictive discrimination, which is defined in this study as the ability to separate those fish likely to be injured (event) from those not (nonevent). An area under the ROC curve of 0.5 indicates no discrimination. Models with an area under an ROC < 0.7 have poor discriminatory capacity; those with areas under the ROC curve of $0.7 \leq \text{ROC} < 0.8$ have an acceptable levels of discrimination; $0.8 \leq \text{ROC} < 0.9$ is considered excellent discrimination; $0.9 \leq \text{ROC} < 1$ is outstanding; an ROC curve area of 1.0 indicates perfect discrimination (Hosmer and Lemeshow 2000; Johnston et al. 2000). Models with ROC areas above 0.80 have been endorsed for individual predictions (Johnston et al. 2000).

In logistic regression, the probability of an outcome is related to a series of potential predictor variables by an equation of the form $\log[p/(1-p)] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$ where p is the probability of the outcome of interest, β_0 is an intercept term, χ_1, χ_2, and χ_3 are the potential predictor variables, and β_1, β_2, and β_3 are coefficients associated with each variable (Tu 1996). Null hypotheses of the effects of individual voltage gradient levels, waveforms, and fish size group were evaluated, using Wald chi-square tests, in the model best representing the empirical data. The estimated coefficients from the best model, as indicated by our selection criterion, were used to estimate odds ratios. The odds ratio is a measure of association approximating how much more likely, or unlikely, it is for the outcome of interest (injury) to be present among fish exposed to one level of a variable than among those exposed to another (e.g., the presence of injury in fish exposed to 60-Hz PDC versus those exposed to DC).

General Model Selection and Inference.—The data from the series of electroshock-induced injury experiments were pooled. Univariate analyses relating size group (S), fish response (R), and fish species (Sp) to injury were conducted. The data set was then split into two size groups, small (≤ 230 mm) and large (≥ 230 mm) and models relating fish size, fish response, mass and species to injury were evaluated. Finally, a mechanistic explanation for the variation in injury rates within and among species was defined and explored.

RESULTS OF EXPERIMENTS

During 1998, 1999, and 2000 electroshock-induced injury data were collected on a total of 2248 fish at various locations within the U.S. (Table 2). Fish from five genera and six species were used. Size and morphology varied considerably among the fishes. Controlled experiments were conducted on chinook salmon *Oncorhynchus tshawytscha*, rainbow trout *O. mykiss*, channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and hybrid striped bass *Morone saxatalis* x *M. chrysops.* All experiments included fish size, electrical waveform, and voltage gradient as treatment variables. Most experiments involved 400 fish, except for those on chinook salmon and channel catfish where fewer fish were available.

Chinook salmon.—Juvenile chinook salmon in two size groups (corresponding to age 0 and age 1) were exposed to uninterrupted DC or 60-Hz PDC (Figure 1) at 0.2, 0.8, or 1.2 V/cm. Twenty chinook salmon were assigned to each of 14 experimental groups, with twelve of the experimental groups defined by combinations of electrical waveform, voltage gradient, and fish size (2 waveforms $\times$ 3 voltage gradients $\times$ 2 size groups). Two groups were designated as controls, one for each size group. The electrical treatments were applied to the test tank electrodes at low, medium, or high voltage levels: 28-54 V (32 $\pm$ 6 V) for low, 96-104 V (100 $\pm$ 2 V) for medium, and 146-160 V (150 $\pm$ 2 V) for the high level.

Table 2.— Location, species, and number of fish used. The fish species, with number used (N), the location of the hatcheries and research centers at which the experiment was conducted, and the dates of the electroshock-induced injury experiments.

Species	Location of Experiment	Date	N
Chinook salmon	Abernathy Fish Technology Center, Abernathy, WA	1-3 July 1998	280
Channel catfish	National Warm-Water Aquaculture Center, Mississippi State University, MS	16-18 Sept 1998	368
Rainbow trout	Alaska Department of Fish and Game, Fort Richardson Hatchery, Anchorage, AK	27-29 July 1999	400
Largemouth bass	Heart of the Hills Research Station, Kerville, TX	2-6 Sept 2000	400
Bluegill	Heart of the Hills Research Station, Kerville, TX	7-9 Sept 2000	400
Hybrid striped bass	North Carolina State University, Pamlico Aquaculture Center, Aurora, NC	10-14 Oct 2000	400

Ambient water conductivity and temperature ranged from 262 to 279 μS/cm (273 ± 7) and temperature from 12.3 – 12.5 °C (12.4 ± 0.1), respectively.

A total of 280 (139 age-0 and 141 age-1) chinook salmon were used in the experiment (Table 2). Age-0 salmon ranged between 84 and 124 mm (mean ± SD; 104 mm ± 8.6) total length and weighed from 4.8 to 16.0 g (9.8 g ± 0.2). The age-1 salmon ranged between 139-193 mm (164 mm ± 8.3) total length and weighed 20.8-71.2 g (36.5 g ± 7.1). There was a statistically significant difference in mean length ($P < 0.001$) and mean weight ($P < 0.001$) between the two age groups.

Hemorrhage Evaluation.—Internal hemorrhage was detected in 30 (12%) of the 240 juvenile chinook salmon exposed to electrical treatment. Most of the hemorrhages that occurred were associated with the vertebral column, 77% were categorized as class 2 and 20% as class 3 in perceived severity. Only 3% of the hemorrhages occurred in the lateral musculature (class 1). One injury was detected in a fish designated as a control, a class 2 hemorrhage in an age-1 fish.

Electrical waveform, size group, and voltage gradient were demonstrated to influence hemorrhage rate in juvenile chinook salmon exposed to electrical treatment (Table 3). Hemorrhage rate, when pooled across voltage gradient and size group, was significantly greater in juvenile chinook salmon exposed to 60-Hz PDC (30/120) compared to DC ($P < 0.001$), as no hemorrhages were detected in salmon exposed to continuous DC. There was a statistically significant difference in hemorrhage rate pooled across voltage gradient and waveform,

Table 3.— Chinook salmon rate of hemorrhage. Rates of hemorrhage among juvenile chinook salmon exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Group comparisons	Hemorrhage Rate	Relative Risk	P- value
Fish size (waveform and voltage gradient pooled)			
Large size vs. small size	23/121 vs. 7/119	3.1 (1.6-8.7)	< 0.001
Waveform (size and voltage gradient pooled)			
60-Hz PDC vs. DC	30/120 vs. 0/120	61 (43-79)	<0.001
Voltage gradient (size and waveform pooled)			
1.2 V/cm vs. 0.2 V/cm	12/80 vs. 4/80	3.0 (1.1-17.0)	0.062
0.8 V/cm vs. 0.2 V/cm	14/80 vs. 4/80	3.5 (1.4-17.0)	0.022
Large fish; 60-Hz PDC			
1.2 V/cm vs. 0.2 V/cm	9/19 vs. 2/21	5.0 (1.7-25.3)	0.012
0.8 V/cm vs. 0.2 V/cm	12/22 vs. 2/21	5.7 (1.9-27.7)	0.003
Small fish; 60-Hz PDC			
1.2 V/cm vs. 0.2 V/cm	3/20 vs. 2/20	1.5 (0.2-9.0)	1.000
0.8 V/cm vs. 0.2 V/cm	2/18 vs. 2/20	1.1 (0.2-7.7)	1.000

between large and small fish ($P < 0.001$); large fish were more likely to have hemorrhages than small fish. Within the large size group, pair-wise comparisons of fish exposed to 60-Hz PDC demonstrated statistically significant differences for hemorrhage rate between salmon exposed to 1.2 V/cm and 0.2 V/cm. ($P = 0.012$) and between those groups exposed to 0.8 V/cm and 0.2 V/cm ($P = 0.003$). No statistically significant difference was noted between the 1.2 V/cm and 0.8 V/cm groups ($P = 0.758$). No statistically significant differences were noted for rates of hemorrhages between the 0.2, 0.8,and 1.2 V/cm groups ($P = 1.000$), among the small fish exposed to 60-Hz PDC.

Vertebral Injury Evaluation.— Radiographic examination revealed vertebral injuries in 8% of the 240 juvenile chinook salmon exposed to electrical treatment (Table 4). Vertebral injury rate was significantly higher in large fish (19/121) compared to the small fish ($P < 0.001$), as no vertebral injuries were detected in any small salmon. The vertebral injuries detected in the large size group of fish were class 1 (53%) or class 2 (47%) in perceived severity. There was no statistically significant difference in vertebral injury rate of large fish exposed to 60-Hz PDC, compared to those exposed to DC ($P = 0.135$). Large juveniles exposed to 60-Hz PDC at 1.2 V/cm had a statistically greater vertebral injury rate than those exposed at 0.2 V/cm ($P = 0.017$). There was no statistically significant difference in the vertebral injury rates of large juvenile

Table 4.— Chinook salmon vertebral injury rate. Rates of vertebral injury among juvenile chinook salmon exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

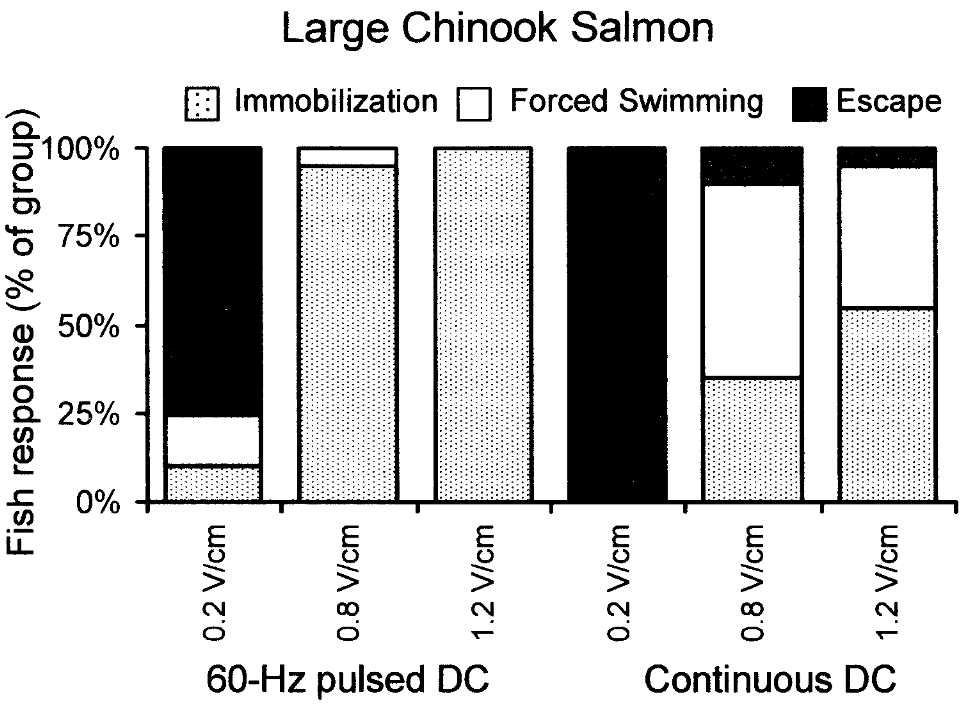
Group comparisons	Injury rate	RR	P-value
Fish size (waveform and voltage gradient pooled)			
Large vs. small	19/121 vs. 0/119	38 (23-54)	< 0.001
Waveform (size and voltage gradient pooled			
60-Hz PDC vs. DC	13/120 vs. 6/120	2.2 (0.9-8.0)	0.150
Voltage gradient (size and waveform pooled)			
1.2 V/cm vs. 0.2 V/cm	9/80 vs. 2/80	4.5 (1.3-23)	0.006
0.8 V/cm vs. 0.2 V/cm	8/80 vs. 2/80	4.0 (1.0-21)	0.098
Large fish			
60-Hz PDC vs. DC	13/62 vs. 6/59	2.1 (0.8-6.7)	0.014
Large fish; 60-Hz PDC			
1.2 V/cm vs. 0.2 V/cm	7/19 vs. 1/21	7.7 (1.8-23)	0.017
0.8 V/cm vs. 0.2 V/cm	5/22 vs. 1/21	4.8 (1.0-16)	0.185
Large fish; DC			
1.2 V/cm vs. 0.2 V/cm	02/20 vs. 1/19	1.9 (0.2-8.6)	1.000
0.8 V/cm vs. 0.2 V/cm	03/20 vs. 1/19	2.9 (0.3-11)	0.605

salmon exposed to 60-Hz PDC at 0.8 V/cm compared to those exposed at 0.2 V/cm. No statistically significant differences were demonstrated in vertebral injury rates for large fish exposed to continuous DC at 1.2 V/cm ($P = 1.000$) or 0.8 V/cm ($P = 0.605$) when compared to 0.2 V/cm (Table 4).

Concurrent Hemorrhage and Vertebral Injury.— Five fish sustained both hemorrhage and vertebral injury, 2% of the salmon exposed to electroshock. Hemorrhages were detected in 26% of those fish having vertebral injury. Vertebral injury was detected in 16% of those fish having hemorrhage.

Induced Behavior and Injury. —The escape response was common in salmon exposed to 0.2 V/cm, regardless of electrical waveform and fish size. The two higher voltage gradients tended to evoke forced swimming and immobilization. Immobilization was the predominant fish response to 1.2 V/cm; whereas, forced-swimming occurred most often in fish exposed to DC (Figure 3).

Evaluation of injury rates among the categories of behavioral responses demonstrated that hemorrhage rate was greater for juvenile chinook salmon that had been immobilized by electroshock compared to those exhibiting escape ($P < 0.001$) or forced swimming ($P = 0.009$) responses. There was no statistically significant difference in hemorrhage rate between the escape response and forced swimming behaviors ($P = 0.607$; Table 5). Although there were no statistically significant differences in vertebral injury rates among the three response categories ($P = 0.068$-1.000), risk of vertebral injury was lower in fish



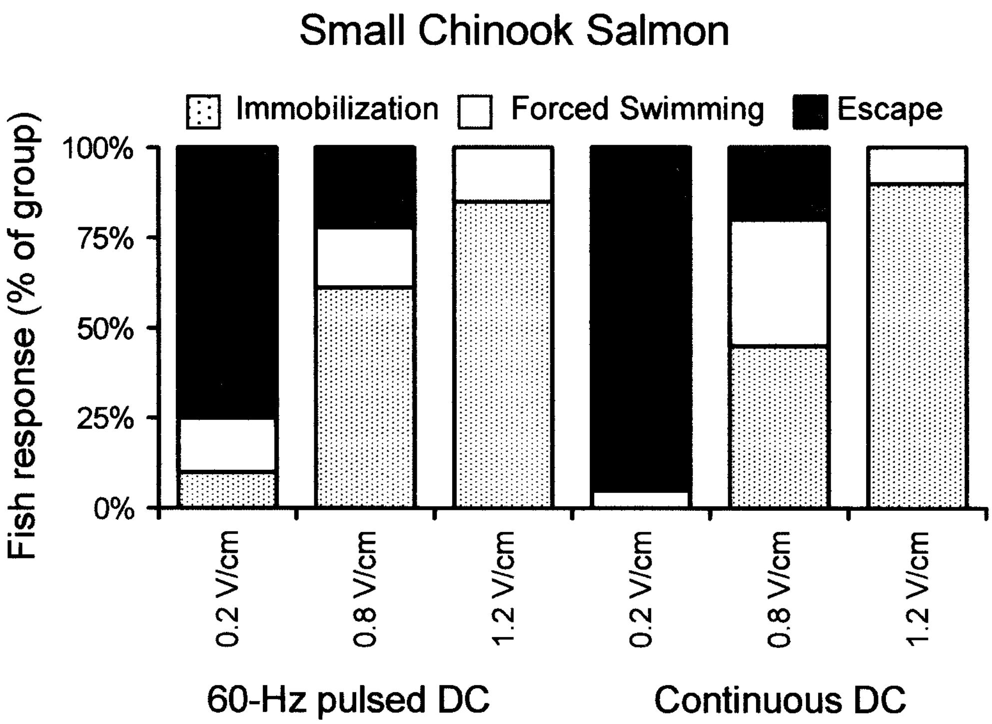


Figure 2. — Chinook salmon behavioral responses. Behavioral responses induced in in juvenile chinook salmon during exposure to various electrical treatments.

Table 5.— Chinook salmon behavioral response and injury. Injury (hemorrhage and vertebral injury) rates among the behavioral responses evoked in juvenile chinook salmon during exposure to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Response comparisons	Injury rate	RR	P-value
	Hemorrhage		
Escape vs. immobilization	2/80 vs. 26/118	0.1 (0.02-0.3)	< 0.001
Escape vs. forced swimming	2/80 vs. 2/42	0.5 (0.1-3.6)	0.607
Forced swimming vs. immobilization	2/42 vs. 26/118	0.2 (0.04-0.6)	0.009
	Vertebral injury		
Escape vs. immobilization	3/80 vs. 14/118	0.3 (0.1-0.9)	0.068
Escape vs. forced swimming	3/80 vs. 2/42	0.8 (0.1-4.7)	1.000
Forced swimming vs. immobilization	2/42 vs. 14/118	0.4 (0.1-1.2)	0.242

demonstrating an escape response than in those that were immobilized (RR = 0.3; 95% CI 0.1-0.9; Table 5).

Injury Model Selection and Evaluation.—No internal hemorrhaging was detected in juvenile chinook salmon exposed to DC. Thus, voltage gradient and salmon size effects were evaluated only for the 120 salmon exposed to 60-Hz PDC. Univariate analysis indicated that fish size (P = 0.003) and voltage gradient (P = 0.030) were significant single predictors of hemorrhage rate in juvenile chinook salmon. The (E, S) model was indicated to be the single best model by the model selection criteria. Comparison of the Akaike weights of the (E, S) model and the next best model, the (S) model, indicated the (E, S) model $(\omega_{(E,S)} = 0.92)$ was about 13 times as likely as the (S) model $(\omega_{(S)} = 0.07)$ for being the best model (Table 6). Voltage gradient (E) and fish size (S) had significant effects in the model (P = 0.024 and 0.002). Hemorrhage was about 5 times more likely in salmon exposed to 60-Hz PDC at 1.2 V/cm (OR = 4.8, 95% CI 1.4-19.4; P = 0.017) or 0.8 V/cm (OR = 5.4, 95% CI 1.6-21.4; P = 0.009) compared to those exposed at 0.2 V/cm. Hemorrhage was about 5 (OR = 4.7, 95% CI 1.8-13.2) times more likely in large salmon compared to small salmon (P = 0.002). The Hosmer-Lemeshow GOF test indicated an adequate fit of the model to the data (P = 0.4867). The (E, S) model had an area under the ROC curve of 0.75 indicating an acceptable level of predictive discrimination.

No vertebral injury was detected in small chinook salmon, thus models were applied only to the large fish. Univariate analysis demonstrated that neither electrical waveform (P = 0.109) nor voltage gradient (P = 0.101) were independently predictive of vertebral injury rate in the large fish. Comparison of Δ_i among the models indicated support for the (W, E) model $(\Delta_{(W,E)} = 0)$, the (E) model $(\Delta_{(E)} = 1.1)$, and the (W) model $(\Delta_{(W)} = 2.6)$ for being best. Akaike weights for the models indicated the (W, E) model was about 1.5 times as likely as the (E) model and about four times as likely as the (W) model (Table 7). Further, predictive discrimination capabilities among the models were relatively weak, but the (W, E) model had an area under the ROC curve of 0.71, at the lower end of the acceptability interval. The (E) and (S) models had areas under the ROC curve less than 0.70, below the level of acceptable predictive discrimination.

The models relating fish reaction to electroshock and injury indicated that evoked response was not independently predictive of hemorrhage (P = 0.202). Evoked response was, however, independently predictive of vertebral injury rate (P = 0.008). Vertebral injuries were about 9 (95% CI 2.1 – 42) times more likely to occur in fish that were immobilized compared to those demonstrating an escape response; those fish exhibiting forced swimming or escape were at similar risk for vertebral injury (OR = 3.0; 95% CI 0.4 – 24). The area under the ROC curve indicated that model (R) had poor discriminatory ability for the occurrence of hemorrhage (0. 69) and vertebral injury (0.65).

Channel catfish.—A total of 368 pond-reared channel catfish from two size groups, designated as large and small, were either exposed to continuous DC,

Table 6.— Chinook salmon model selection (hemorrhage). Summary of selection statistics for models relating the experimental variables to the occurrence of hemorrhages in juvenile chinook salmon. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Model	Δ_i	ω_i	ROC	GOF
(E, S)	0	0.92	0.75	0.49
(S)	5.3	0.07	0.67	—
(E)	9.0	0.01	0.65	1.00

Table 7.— Chinook salmon model selection (vertebral injury). Summary of selection statistics for models relating the experimental variables to vertebral injury in juvenile chinook salmon. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Model	Δ_i	ω_i	ROC	GOF
(W, E)	0	0.54	0.71	0.82
(E)	1.1	0.32	0.65	1.00
(W)	2.6	0.15	0.60	—

15-Hz PDC, or 60-Hz PDC at 0.2, 0.5, or 1.2 V/cm, or were used as controls (Table 2). Twenty fish were assigned to each of 18 electrical treatment groups, with the groups defined by combinations of waveform, voltage gradient, and fish size (3 waveforms × 3 voltage gradients × 2 size groups). Two additional groups, one for each size group, were designated as controls. A shortage of fish led to unequal numbers in the experimental groups. The electrical treatments were applied to the test tank electrodes at 20 volts (20 ± 1.1 V), 60 V (60 V ± 2 V), or 150 V (150 V ± 2 V).

There was a significant difference in mean total length ($P < 0.001$) and weight ($\underline{P} < 0.001$) of the fish in the large and small size groups, as indicated by the two sample t-tests. Catfish in the large size group were 272 to 485 mm (356 mm ± 39), those in the small size group 105 to 256 mm (157 mm ± 23). Weights ranged from 153 to 1129 g (365 g ± 143) in the large size group of fish and from 7 to 150 g (25 g ± 15) in the small size group.

There were 185 catfish in the large size group and 183 in the small size group. Nineteen of the large and 17 of the small catfish were designated as control fish. A total of 332 catfish were exposed to electrical treatment. Injury evaluation data from bilateral filleting of one large fish, subjected to DC at a field intensity of 0.2 V/cm, was mistakenly not recorded. Consequently, this fish was dropped from the hemorrhage rate analysis. Radiographs of adequate diagnostic quality for evaluation of vertebral injury were obtained for 280 of the 332 catfish (84%) that were subjected to electrical treatments. This loss of information was random with respect to experimental design. Vertebral injury could not be evaluated on one control fish from the large size group. No internal hemorrhages or vertebral injuries were detected in any catfish from the control groups.

Hemorrhage Evaluation.— Among the 331 electroshocked catfish examined for injury via bilateral filleting, 18% (58/331) displayed evidence of injury. Hemorrhages, when they occurred, were associated with the vertebral column. When pooled across electric waveforms and voltage gradients, hemorrhage rate was significantly greater in large size catfish (34%) compared to small catfish (1%; $P < 0.001$; Table 8). Hemorrhages in catfish from the large size group were predominantly (61%) class 2 in perceived severity, with the remaining hemorrhages (39%) being class 3. Two catfish from the small size group had hemorrhages that were rated as class 3; both had been exposed to DC, one at 0.2 V/cm and the other at 0.5 V/cm.

The remaining hemorrhage rate analysis focused on the large catfish (Table 8). Hemorrhage rate differed significantly among the three waveforms, when pooled across voltage gradient ($P = 0.001$). Risk of hemorrhage was highest among large catfish exposed to 60-Hz PDC (48%) and lowest among those exposed to 15-Hz PDC (13%). Hemorrhage rate differed significantly between 60-Hz PDC and 15-Hz PDC ($P = 0.000$), but no statistically significant difference was noted between 60-Hz PDC and DC (42%; $P = 0.564$).

Table 8.— Channel catfish rate of hemorrhage. Rates of hemorrhage among channel catfish exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Group comparisons	Hemorrhage rate	RR	P-value
Fish size (waveform and voltage gradient pooled)			
Large size vs. small size	56/165 vs. 2/164	28 (7.0-114)	< 0.001
Large fish (Voltage gradient pooled)			
60-Hz PDC vs. DC	27/56 vs. 22/53	1.2 (0.7-1.8)	0.564
15-Hz PDC vs. DC	7/56 vs. 22/53	0.3 (0.1-0.7)	0.001
60-Hz PDC vs. 15-Hz PDC	27/56 vs. 7/56	3.9 (2.0-11.0)	< 0.001
Large fish (waveform pooled)			
1.2 V/cm vs. 0.2 V/cm	25/56 vs. 8/54	3.0 (1.7-8.0)	< 0.001
0.5 V/cm vs. 0.2 V/cm	23/55 vs. 8/54	2.8 (1.5-7.9)	0.003
1.2 V/cm vs. 0.5 V/cm	25/56 vs. 23/55	1.1 (0.7-1.7)	0.849
Large fish; 60-Hz PDC			
1.2 V/cm vs. 0.2 V/cm	10/19 vs. 6/20	1.8 (0.8-5.3)	0.200
0.5 V/cm vs. 0.2 V/cm	11/17 vs. 6/20	2.2 (1.1-5.9)	0.050
1.2 V/cm vs. 0.5 V/cm	10/19 vs. 11/17	0.8 (0.5-1.5)	0.516
Large size fish; 15-Hz PDC			
1.2 V/cm vs. 0.2 V/cm	5/18 vs. 0/20	12 (3.3-21.0)	0.017
0.5 V/cm vs. 0.2 V/cm	2/16 vs. 0/20	6.2 (1.2-13.6)	0.218
1.2 V/cm vs. 0.5 V/cm	5/18 vs. 2/16	2.5 (0.4-13.2)	0.402
Large size fish; 15-Hz DC			
1.2 V/cm vs. 0.2 V/cm	10/19 vs. 2/14	3.7 (1.3-19)	0.033
0.5 V/cm vs. 0.2 V/cm	10/20 vs. 2/14	3.5 (1.1-16)	0.066
1.2 V/cm vs. 0.5 V/cm	10/19 vs. 10/20	1.0 (0.6-2.1)	1.000

Furthermore, hemorrhages were significantly less likely in catfish exposed to 15-Hz PDC than in those exposed to DC ($P = 0.001$). Hemorrhage rate varied significantly among the voltage gradients, when pooled across the electrical waveforms ($P = 0.001$). Among the large catfish exposed to 1.2 V/cm, 45% had a hemorrhage compared to 42% of those exposed to 0.5 V/cm ($P = 0.849$), and 15% of those exposed to 0.2 V/cm ($P = 0.001$). The large size fish exposed to 0.5 V/cm were also more likely to have broken blood vessels than those exposed to 0.2 V/cm ($P = 0.003$; Table 8). Hemorrhage rate associated with voltage gradient varied significantly in fish exposed to 15-Hz PDC ($P = 0.037$). Conversely, no statistically significant differences in hemorrhage rate were detected among the voltage gradients in catfish exposed to 60-Hz PDC ($P = 0.102$) or DC ($P = 0.057$). Pair-wise comparison of hemorrhage rates of catfish exposed to 15-Hz PDC, among the voltage gradients, demonstrated a statistically significant difference between 1.2 V/cm and 0.2 V/cm ($P = 0.017$), but not between 1.2 V/cm and 0.5 V/cm ($P = 0.402$) or between 0.5 V/cm and 0.2 V/cm ($P = 0.218$; Table 8).

Vertebral Injury Evaluation.—Among the 280 catfish evaluated for electroshock-induced injury radiographically, 47 (17%) had vertebral injuries. Catfish within the large size group accounted for 98% of the vertebral injuries detected. Vertebral injury rate differed significantly between large and small sized catfish ($P < 0.001$). Most vertebral injuries within the large size group of catfish were rated as class 2 (52%) or class 3 (39%) in perceived severity, exceptions were the 9%

with class 1 injury ratings. The single catfish in the small size group with a vertebral injury, which had been exposed to 60-Hz PDC at a field intensity of 0.2 V/cm, had a class 3 rating. Further analysis of vertebral injury rate, with regard to waveform and voltage gradient effects, focused exclusively on the large catfish.

Vertebral injury rate, pooled over the voltage gradients, varied significantly among the electrical waveforms evaluated ($P = 0.001$; Table 9). The highest rate of vertebral injury occurred in catfish exposed to 60-Hz PDC (44%), the lowest in those exposed to 15-Hz PDC (8%), a statistically significant difference ($P < 0.001$). Similarly, vertebral injury rate was significantly lower in catfish exposed to 15-Hz PDC (8%) compared to those exposed to DC (36%; $P = 0.001$). No statistically significant difference in vertebral injury rate was noted between catfish exposed to 60-Hz PDC and DC ($P = 0.550$).

Vertebral injury rate varied significantly among the voltage gradients evaluated ($P = 0.020$), when pooled across the electrical waveforms. There was no significant difference in risk for vertebral injury in large catfish exposed to 1.2 V/cm (39%) compared to those exposed to 0.5 V/cm (32%; $P = 0.549$). Nor was there a statistically significant difference in vertebral injury rate between catfish exposed to 0.5 V/cm and 0.2 V/cm (15%; $P = 0.060$). Large channel catfish exposed to 1.2 V/cm were at significantly higher risk for vertebral injury than those subjected to 0.2 V/cm ($P = 0.008$; Table 9). There was a trend for hemorrhage rate to increase with voltage gradient, regardless of waveform, but

Table 9.—Channel catfish vertebral injury rate. Rates of vertebral injury among channel catfish exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Group comparisons	Vertebral injury rate	RR	P-value
Size (waveform and voltage gradient pooled)			
Large size vs. small size	46/157 vs. 1/123	36 (11-87)	< 0.001
Large fish (voltage gradient pooled)			
60-Hz PDC vs. 15-Hz PDC	24/55 vs. 4/52	5.7 (2.7-26)	< 0.001
15-Hz PDC vs. DC	4/52 vs. 18/50	0.2 (0.0-0.5)	0.001
60-Hz vs. DC	24/55 vs. 18/50	1.2 (0.7-2.0)	0.550
Large fish (waveform pooled)			
1.2 V/cm vs. 0.5 V/cm	22/56 vs. 17/53	1.2 (0.8-2.2)	0.549
1.2 V/cm vs. 0.2 V/cm	22/56 vs. 7/48	2.7 (1.4-8.1)	0.008
0.5 V/cm vs. 0.2 V/cm	17/53 vs. 7/48	2.2 (1.1-6.0)	0.060

the trend was not statistically significant: 60-Hz PDC ($P = 0.050$); 15-Hz PDC ($P = 0.355$); and DC ($P = 0.088$; Table 9).

Concurrent Hemorrhage and Vertebral Injury.—Injury was detected in five fish by both methods. Hemorrhage was detected in 26% (5/19) of the channel catfish that had vertebral injuries. Vertebral injury was detected in 17% (5/30) of the catfish that had hemorrhages.

Induced behavior and injury.—A total of 332 catfish were exposed to electrical treatment: 166 large catfish and 166 small. Behaviors favorable for capture of catfish were evoked from 80% of the large catfish, immobilization from 52% and forced swimming from 28%. Behaviors favorable for capture were elicited from 46% of the small catfish: immobilization from 36% and forced swimming behaviors from 10% (Figure 4).

The two small channel catfish with hemorrhages exhibited the escape response during exposure to DC at 0.2 V/cm. One vertebral injury was detected in a small catfish. This fish had displayed the escape reaction during exposure to 60-Hz PDC at 0.2 V/cm.

Hemorrhage rate was greater in large channel catfish that had been immobilized (54%; 47 of 86) compared to those displaying forced swimming (13%; 6 of 46; RR = 4.2; 95% CI 2.2-13.4; $P < 0.001$) or escape responses (9%; 3 of 33; RR = 6.0; 95% CI 2.6-35.6; $P < 0.001$). There was no statistically significant difference in hemorrhage rate between catfish displaying forced swimming compared to those exhibiting an escape response ($P = 0.727$).

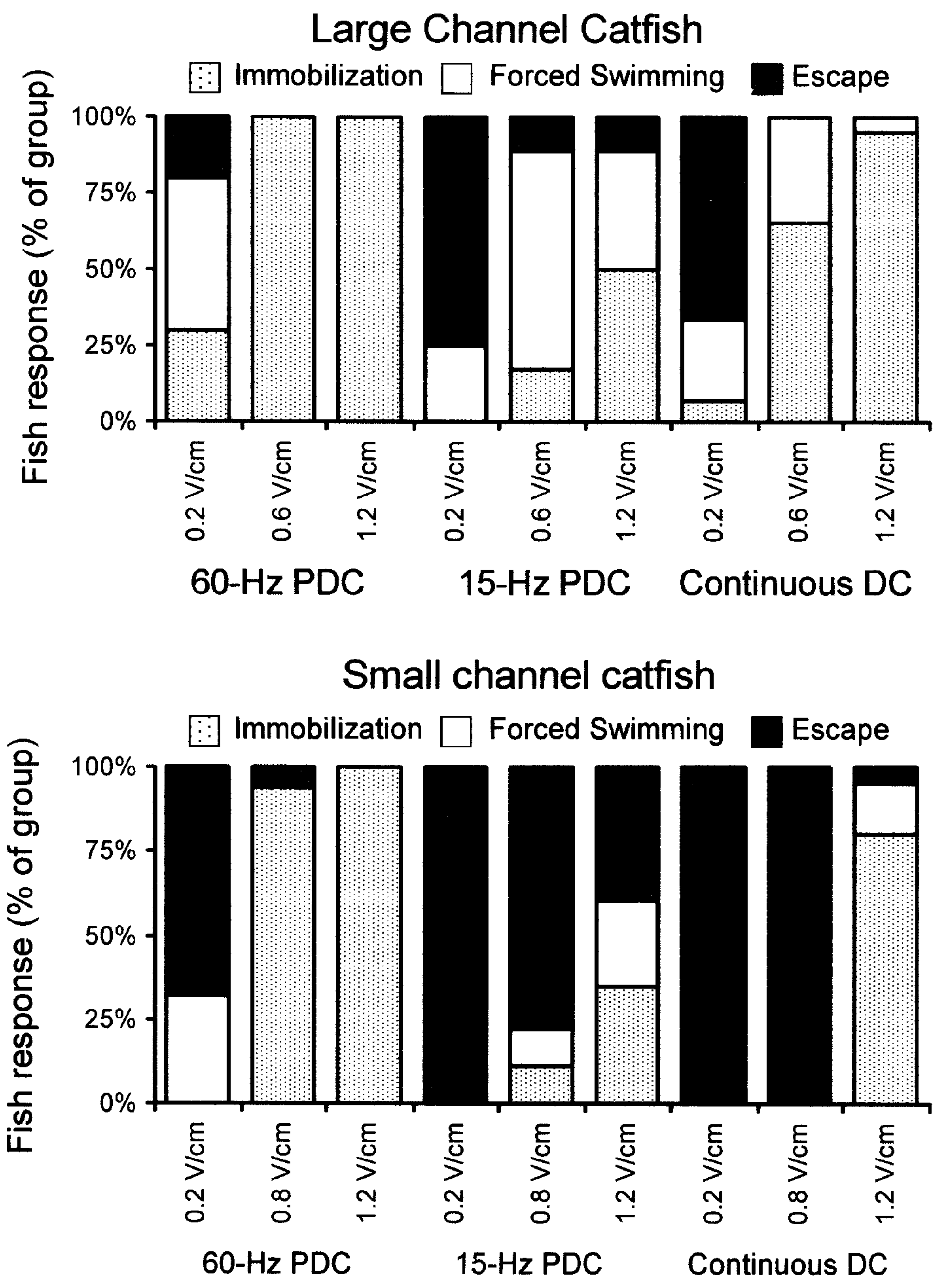


Figure 3. — Channel catfish behavioral responses. Behavioral responses induced in channel catfish during exposure to various electrical treatments.

Table 10.—Channel catfish model selection (hemorrhage). Summary of selection statistics for models relating the experimental variables to the occurrence of hemorrhage in channel catfish The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Model	Δ_i	ω_i	ROC	GOF
(W, V)	0	0.54	0.71	0.82
(V)	1.1	0.32	0.65	1.00
(W)	2.6	0.15	0.60	—

Vertebral injury rate for large channel catfish that were immobilized (48%; 40 of 83) varied significantly compared to those displaying forced swimming behaviors (9%; 4 of 44; RR = 5.3; 95% CI 2.6-22.3; $P < 0.001$) and escape (7%; 2 of 30; RR = 7.2; 95% CI 2.7-32.8; $P < 0.001$). There was no statically significant difference in vertebral injury rate between those catfish demonstrating a forced swimming behavior compared to those displaying an escape response ($P = 1.000$).

Injury Model Selection and Evaluation.— Fish size (S), electric field intensity (E), and electrical waveform (W) were demonstrated to be independently predictive of hemorrhage in channel catfish ($P = 0.001$). The main effects model (W, E, S) was ranked the highest of the models evaluated, indicating that it was the best model (Table 10). Significant field intensity, size group, and waveform effects were demonstrated in the model, as indicated by Wald chi-square tests ($P < 0.01$).

Channel catfish in the large size group were 54 times (95% CI 13-230) as likely to suffer hemorrhage than those in the small size group, as indicated by the model. Channel catfish subjected to 60-Hz PDC or DC had similar risk for muscle injury ($P = 0.52$). However, channel catfish subjected to 15-Hz PDC were about five times less likely to suffer a hemorrhage than those fish subjected to 60-Hz PDC or DC ($P < 0.01$, OR = 0.18 (95% CI 0.07-0.48). Significant voltage effects were indicated in the model; catfish subjected to 1.2 V/cm (OR = 4.2; 95% CI 1.7-10.7; $P < 0.00$) or 0.5 V/cm (OR = 4.2; 95% CI 1.6-10.6; $P < 0.01$) were more likely to suffer hemorrhage relative to those subjected to 0.2 V/cm. The ROC curve constructed for the predictive model of muscle injury indicated an area under the curve of 0.88, indicating that the model had very good discriminatory ability.

Univariate analysis on the 280 catfish evaluated for vertebral injury, indicated size group (S) and electrical waveform to be independently predictive of vertebral injury ($P < 0.01$), whereas, field intensity (E) was not statistically significant ($P = 0.06$). However, field intensity was independently predictive of vertebral injury within the large size group ($P = 0.02$). The (W, E, S) instrument was the best model for vertebral injury in channel catfish, as indicated by the selection criterion (Table 11). Significant waveform, field intensity, and fish size group effects were demonstrated in the model, as indicated by Wald chi-square tests ($P < 0.05$). Catfish subjected to 1.2 V/cm were at higher risk than those exposed to 0.2 V/cm (OR = 3.7; 95% CI 1.4-10.4; $P < 0.01$). Catfish exposed to 15-Hz PDC were at lower risk for injury than those subjected to 60-Hz PDC (OR = 0.09; 95% CI 0.03-0.3; $P < 0.01$) and DC (OR = 0. 15; 0.04-0.46; $P < 0.01$). Catfish in the large size group were more likely to have injured vertebrae than those in the small size group (OR = 60.5; 95% CI 12.5-∞; $P = 0.03$). However, comparisons of risk for vertebral injury between catfish subjected to 1.2 V/cm and 0.5 V/cm ($P = 0.42$), 0.5 V/cm and 0.2 V/cm ($P = 0.06$), and 60-Hz PDC and DC ($P = 0.21$), revealed no significant differences in risk. The ROC curve indicated

Table 11.— Channel catfish model selection (vertebral injury). Summary of selection statistics for models relating the experimental variables to vertebral injury in channel catfish. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Models	Δ_i	ω_i	ROC	GOF
(W, S)	4	0.12	0.85	0.92
(E, S)	19	0.00	0.81	0.2
(S)	23	0.00	0.75	—
(W, E)	52	0.00	0.72	0.98
(W)	55	0.00	0.68	0.99
(E)	70	0.00	0.60	0.99

that the model had very good discriminatory capabilities; the area under the curve was 0.87.The models relating evoked response to injury indicated fish response to be independently predictive of vertebral injury and hemorrhage in channel catfish (P = 0.00). Hemorrhages were 11 (95% CI 4.3 – 29.3) times more likely to occur in fish that were immobilized compared to those demonstrating an escape response; those catfish exhibiting forced swimming or escape were at similar risk for hemorrhage (OR = 2.5; 95% CI 0.7 – 8.7). Immobilized fish were 4.4 times more likely to have a hemorrhage than those demonstrating a forced swimming response. Fish that were immobilized were 6.3 times more likely to suffer vertebral injury than those exhibiting forced swimming and 15.2 times (95% CI 4.5 – 51) more likely than those with an escape response. Fish exhibiting forced swimming were no more likely to suffer vertebral injury than those demonstrating an escape response (OR = 2.4; 95% CI 0.5 – 11.2). Each model had acceptable discriminatory ability, the model relating evoked response to vertebral injury had an area under the ROC curve of 0.75 and the model relating response to mytomal hemorrhage had an area under the ROC curve of 0.74.

Rainbow trout.—A total of 401 hatchery-reared rainbow trout from two size groups, designated as large and small, were exposed to continuous DC, 15 Hz PDC, or 15-Hz GTB), at 0.4, 0.8, or 1.9 V/cm, or were used as controls. The electrical treatments were applied to the test tank electrodes at 50 V (± 2 V), 100

V (± 2 V), or 239 V (± 3.0 V). Water temperature in the test tank ranged from 11.3 to 11.9 C and ambient conductivity from 131 to 137 μS/cm.

Rainbow trout in the small size group were 103 to 186 mm (145 mm ± 15) total length and weighed 10 to 57 g (32 g ± 9). Fish in the large size group were 237 to 480 mm (324 mm ± 39) total length and weighed 162-1050 g (343 g ± 129). Mean length ($P = 0.0001$) and mean weight ($P = 0.0001$) differed significantly between the two size groups, as indicated by the two sample t-tests.

Hemorrhage Evaluation.—An 11% (39/360) rate of internal hemorrhage was demonstrated in the bilateral filleting evaluations of the electroshocked rainbow trout. The majority of the hemorrhages were associated with the backbone, 30% were class 2 and 49% were class 3 in perceived severity. The remaining hemorrhages (21%) were located in the dorsal or lateral musculature (i.e., class 1 in perceived severity). Bilateral filleting of the control fish detected hemorrhage associated with the backbone in one trout from the large size group.

When hemorrhage rate was pooled across waveform and voltage gradient, 21% of the rainbow trout in the large size group had hemorrhages compared to 1% of the small size group ($P < 0.001$; Table 12). One of the two small rainbow trout that had internal hemorrhage had been exposed to 15-Hz PDC, the other to DC. Both had been exposed to 1.9 V/cm. Hemorrhage rate differed significantly among the electrical waveforms, when pooled across size group and voltage gradient ($P = 0.011$). There was no statistically significant

Table 12.— Rainbow trout rate of hemorrhage. Rates of hemorrhage among rainbow trout exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values for each comparison.

Experimental group	Hemorrhage Rate	*RR*	P-value
Fish size (waveform and voltage gradient pooled)			
Large vs. small	37/180 vs. 2/180	18.5 (6.6-83)	< 0.001
Waveform (size and voltage gradient pooled)			
15-Hz PDC vs. DC	7/120 vs. 21/120	0.3 (0.1-0.7)	0.007
15-Hz GTB vs. DC	11/120 vs. 21/120	0.5 (0.2-1.0)	0.086
Voltage gradient (size and waveform pooled)			
1.9 V/cm vs. 0.4 V/cm	14/120 vs. 10/120	1.4 (0.6-3.5)	0.519
0.8 V/cm vs. 0.4 V/cm	15/120 vs. 10/120	1.5 (0.7-3.6)	0.299
Large fish (voltage gradient pooled)			
15-Hz PDC vs. DC	6/60 vs. 20/60	0.3 (0.1-0.6)	0.003
15-Hz GTB vs. DC	11/60 vs. 20/60	0.6 (0.3-1.0)	0.094
Large fish; 15 Hz PDC			
1.9 V/cm vs. 0.4 V/cm	2/20 vs. 3/20	0.7 (0.1-5.0)	1.000
0.8 V/cm vs. 0.4 V/cm	1/20 vs. 3/20	0.3 (0.1-3.0)	0.605
Large fish; 15-Hz GTB			
1.9 V/cm vs. 0.4 V/cm	5/20 vs. 1/20	5.0 (1.0-17)	0.182
0.8 V/cm vs. 0.4 V/cm	5/20 vs. 1/20	5.0 (1.0-17)	0.182
Large fish; DC			
1.9 V/cm vs. 0.4 V/cm	5/20 vs. 6/20	0.8 (0.2-2.6)	1.000
0.8 V/cm vs. 0.4 V/cm	9/20 vs. 6/20	1.5 (0.6-4.5)	0.514

correlation between voltage gradient and hemorrhage rate when pooled across electrical waveform and fish size group (P = 0.407). Because hemorrhage rate was very low in the small size group, the remaining statistical analyses were focused on the large size group.

Hemorrhage rate varied by electrical waveform, within the large size group, when pooled across voltage gradient (P = 0.006). Trout exposed to DC had the highest hemorrhage rate (Table 12). Hemorrhage rate was significantly less in trout exposed to 15-Hz DC compared to those exposed to DC (6%; RR = 0.3; P = 0.007). Trout exposed to 15-Hz GTB (9%; RR = 0.5) were also at less risk for hemorrhage than those exposed to DC, but the difference was not statistically significant (P = 0.086).

When the hemorrhage rates associated with voltage gradient within the large size group were pooled across waveform, no statistically significant correlation was demonstrated (P = 0.652). The lack of statistically significant correlation in risk of hemorrhage among the voltage gradients persisted when stratified by waveform (Table 12).

Vertebral Injury Evaluation.— Radiographs of diagnostic quality were obtained for 93% (334/360) of the 360 rainbow trout exposed to electroshock. Vertebral injury was detected in 13% (45/334) of these fish. The majority (40%) of the vertebral injuries were categorized as class 2 (i.e., misalignment of vertebrae) in perceived severity. The remaining vertebral injuries were compression injuries (class 1; 36%) or fractures (class 3; 24%). Among control fish, radiographs of

Table 13.— Rainbow trout vertebral injury rate. Rates of vertebral injury among large rainbow trout exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values for each comparison.

Group comparisons	Vertebral injury rate	RR	P-value
Waveform (voltage gradient pooled)			
15-Hz PDC vs. DC	9/55 vs. 22/55	0.4 (0.2-0.8)	0.010
15-Hz GTB vs. DC	8/57 vs. 22/55	0.4 (0.1-0.7)	0.002
Voltage gradient (waveform pooled)			
1.9 V/cm vs. 0.4 V/cm	12/59 vs. 9/53	1.2 (0.5-3.0)	0.809
0.8 V/cm vs. 0.4 V/cm	18/55 vs. 9/53	1.9 (1.0-4.4)	0.076
15-Hz PDC			
1.9 V/cm vs. 0.4 V/cm	3/20 vs. 1/17	2.5 (0.3-11.1)	0.609
0.8 V/cm vs. 0.4 V/cm	5/18 vs. 1/17	4.7 (0.9-16.1)	0.177
15-Hz GTB			
1.9 V/cm vs. 0.4 V/cm	3/20 vs. 3/19	1.0 (0.2-6.6)	1.000
0.8 V/cm vs. 0.4 V/cm	2/18 vs. 3/19	0.7 (0.1-5.2)	1.000
DC			
1.9 V/cm vs. 0.4 V/cm	6/19 vs. 5/17	1.0 (0.4-4.0)	1.000
0.8 V/cm vs. 0.4 V/cm	11/19 vs. 5/17	2.0 (0.9-8.1)	0.106

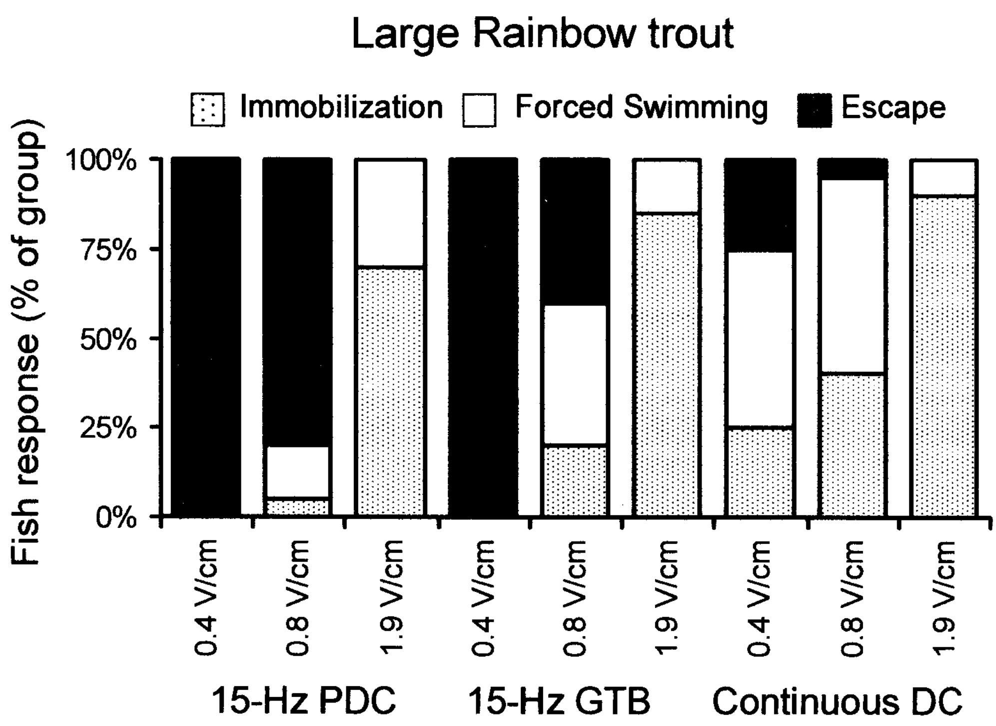
diagnostic quality were obtained for 19 of the 20 small fish and 18 of the 20 of the large fish. No vertebral injury was detected in the control fish. Vertebral injury rate differed significantly between trout in the large size group and those in the small size group when pooled across electrical waveform and voltage gradient (23% versus 4%; RR = 6.5; $P < 0.001$). When pooled across size group and voltage gradient, vertebral injury rate differed by electrical waveform: 11/109 (10%) for 15-Hz PDC; 9/114 (8%) for 15-Hz GTB DC; and 25/111 (23%) for DC ($P = 0.003$).

Vertebral injury rate of large rainbow trout varied significantly among the three electrical waveforms within the large size group ($P = 0.003$; Table 13), when pooled across the voltage gradients. Risk of injury was highest among those exposed to DC. No statistically significant differences in the vertebral injury rates of small rainbow trout was detected among the waveforms, when pooled across the voltage gradients: 2/54 (4%) for 15-Hz PDC; 1/57 (2%) for 15-Hz GTB; and 3/56 (5%) for DC ($P = 0.635$). There was no evidence of a significant correlation between voltage gradient and vertebral injury rate when pooled across waveform and size group ($P = 0.407$; Table 13). This lack of correlation between voltage gradient and vertebral injury persisted for vertebral injury rate pooled across waveform and controlling for size group ($P = 0.591$), and continued when controlling for waveform and size group ($P = 0.581$).

Concurrent Hemorrhage and Vertebral Injury.—Overall, injury, either internal hemorrhage or vertebral damage, was detected in 20% (66/336) of the rainbow trout. Often, injury was detected using one method, but not the other. Hemorrhage was detected in 40% (18/45) of the trout with vertebral injury. Vertebral injury was detected in 48% (18/37) of those trout with a positive hemorrhage status (radiographs were unavailable for two trout that had hemorrhages).

Induced Behavior and Injury.—Overall, 23% of the 360 electroshocked rainbow trout were immobilized and 18% exhibited forced swimming behaviors. Escape was demonstrated by the remaining 58% of the trout. Generally, the evoked responses were more severe in the large rainbow trout. Immobilization was evoked in 10%, forced swimming in 13%, and escape reactions in 77% of the 180 small rainbow trout receiving electrical treatment (Figure 4). In comparison, 37% of the large rainbow trout were immobilized, 24% exhibited galvanotaxis or psuedo-forced swimming, and 39% reacted to electrical treatment with an escape response.

Hemorrhage was detected in two small rainbow trout (9%) that reacted to electrical treatment with forced swimming. Vertebral injury rate did not vary significantly among the behavioral responses of small trout (P = 0.103): 2/16 (13%) for those immobilized, 0/22 (0%) for those reacting with forced swimming, and 4/129 (3%) for those exhibiting escape responses. No statistically significant trend was evident between vertebral injury rate and the behavioral responses (P = 0. 181) of the small sized trout (Table 14).



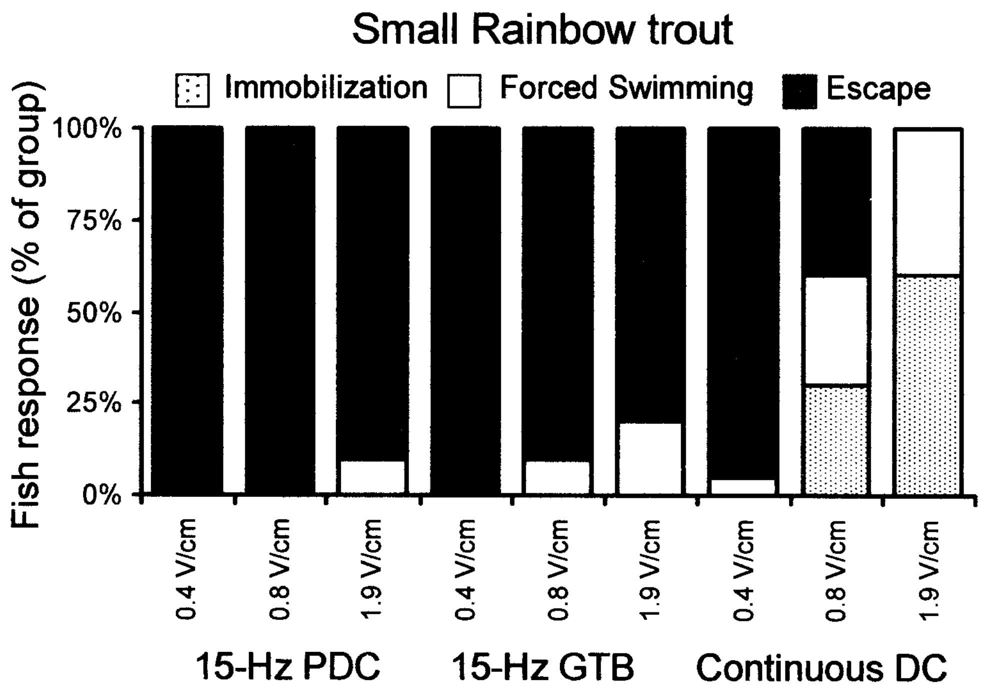


Figure 4. — Rainbow trout behavioral responses. Behavioral responses induced in rainbow trout during exposure to various electrical treatments.

Table 14.— Rainbow trout behavioral response and injury. Injury rate among the behavioral responses of large rainbow trout exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Group comparison	Hemorrhage rate	RR	P-value
Escape vs. immobilization	7/70 vs. 20/67	0.3 (0.1-0.7)	0.004
Escape vs. forced swimming	7/70 vs. 10/43	0.4 (0.2-1.1)	0.064
Forced swimming vs. immobilization	10/43 vs. 20/67	0.8 (0.4-1.5)	0.515

Hemorrhage rate differed significantly among the behavioral responses of the large rainbow trout (P = 0.015; Table 14). Further, there was statistical evidence of a trend for reduction in hemorrhage rate with less severe behavioral responses: 30% of the immobilized fish, 23% of those demonstrating forced swimming, and 10% of those with escape responses (P = 0.004). Conversely, there was no significant difference in vertebral injury rates among the behavioral responses: 18/64 (28%) for immobilization; 11/41 (27%) for forced swimming; and 10/62 (16%) for escape responses (P = 0.237). No statistical evidence of a trend in vertebral injury rate associated with the behavioral responses of large rainbow trout was evident (P = 0.113).

Injury Model Selection and Evaluation.—Overall, 11% of the 360 rainbow trout had hemorrhages. Univariate analysis demonstrated that rainbow trout size (P < 0.001) and electrical waveform (P = 0.015) were statistically significant single predictors of hemorrhage in rainbow trout. Voltage gradient was not statistically significant as a univariate (P = 0.550). The rainbow trout size model had an area under the ROC curve of 0.75, an acceptable level of discrimination, greater than either of the other two models.

The (W, S) model had the lowest AIC value of the models within the candidate set (Table 15). Some support was demonstrated for the (W, S, E), and (S) models as being the best, by Δ; the (W, S, E) model had a Δ value of 3, the univariate (S) model had a Δ value of 6. Comparison of Akaike weights between the (W, S) and (S) models provided strong evidence that the (S) model was

Table 15.— Rainbow trout model selection (hemorrhage). Summary of selection statistics for models relating the experimental variables to the occurrence of hemorrhage in rainbow trout. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Models	Δ_i	ω_i	ROC	P- value
(W, S)	0	0.74	0.82	0.69
(W, S, E)	3	0.21	0.83	0.51
(S)	6	0.04	0.75	—
(S, E)	8	0.01	0.78	0.43
(W)	41	0.00	0.63	1.00
(W, V)	44	0.00	0.65	0.73
(V)	49	0.00	0.55	1.00

unlikely to be the best model. The (W, S) model was 18.5 times more likely than the (S) model to be the single best. The Akaike weights of the two models indicated the (W, S) model was 3.5 times more likely to be the best, compared to the (W, S, E) model. Because no statistically significant voltage gradient effects were demonstrated in the (W, S, E) model, it was dropped from contention as the best.

The (W, S) model was the most plausible model for hemorrhage outcome. There was strong evidence for electrical waveform (P = 0.010) and rainbow trout size (P < 0.001) effects in the model. Large rainbow trout were at statistically significant greater risk for hemorrhage than small rainbow trout (OR = 25, 95% CI 7.3-153; P < 0.001). Rainbow trout exposed to 15-Hz PDC were about four times less likely to have hemorrhages than those exposed to DC, a statistically significant difference (OR = 0.255, 95% CI 0.1-0.6; P = 0.004). Similarly, rainbow trout exposed to the 15-Hz GTB were about two times less likely to demonstrate hemorrhages than those exposed to DC (OR = 0.429, 95% CI 0.2-1.0; P = 0.046). There was no statistically significant difference in hemorrhage rate between rainbow trout exposed to 15-Hz PDC and those exposed to the 15-Hz GTB waveform (P = 0.314). The (W, S) model had excellent discrimination for prediction of rainbow trout hemorrhage, as indicated by the ROC area of 0.82. There was insufficient evidence to reject the hypothesis that the model fit the data, as indicated by the Hosmer and Lemeshow goodness-of-fit test (P = 0.690).

Of the 334 rainbow trout evaluated for injury via radiography, 14% had vertebral damage. Univariate analysis demonstrated electrical waveform ($P = 0.004$) and rainbow trout size ($P < 0.001$) were statistically significant single predictors of rainbow trout vertebral injury. Voltage gradient was not ($P = 0.316$). The (S) model, which had the greatest ROC (0.71) area of the three univariates, was the only univariate model with an acceptable level of predictive discrimination.

The (W, S) model had the lowest AIC value within the candidate set of models. Comparison of model Δ and Akaike weights indicated the (W, E, S) model should also be considered for inference, as the Δ for the (W, S, E) model was 2 and the (W, S) model was only about two times as likely to be the single best model (Table 16). Analysis of effects in the (W, S, E) demonstrated voltage gradient was not statistically significant in the model. Thus, the (W, S, E) model was discarded in favor of the (W, S) model being the single best model of those evaluated.

Significant electrical waveform ($P = 0.002$) and rainbow trout size ($P < 0.001$) effects were demonstrated in the (W, S) model. Rainbow trout exposed to 15-Hz PDC (OR = 0.3, 95% CI 0.2-0.7; $P = 0.009$) or to the 15-Hz GTB waveform (OR = 0.3, 95% CI 0.1-0.6; $P = 0.002$) were significantly less likely to suffer vertebral injury than those exposed to DC. There was no statistically significant difference in risk of vertebral injury between trout exposed to DC pulsed at 15-Hz and those exposed to the 15-Hz gated burst waveform ($P = 0.567$). Large

Table 16.— Rainbow trout model selection (vertebral injury). Summary of selection statistics for models relating the experimental variables to vertebral injury in rainbow trout. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Models	Δ_i	ω_i	ROC	GOF
(W, S)	0	0.68	0.78	0.95
(W, S, E)	2	0.31	0.79	0.96
(S)	9	0.01	0.71	—
(S, E)	10	0.00	0.75	0.64
(W)	30	0.00	0.64	1.00
(W, E)	32	0.00	0.67	1.00
(V)	39	0.00	0.57	1.00

rainbow trout were significantly more likely to suffer vertebral injury than small trout (OR = 9, 95% CI 4-24; $P < 0.001$). The Hosmer and Lemeshow goodness-of-fit test failed to reject the hypothesis that the model fit the data well ($P = 0.954$). The (W, S) model had an ROC area of 0.78, indicating the model had an acceptable level of predictive discrimination.

The models relating evoked response to injury indicated fish response to be independently predictive of hemorrhage ($P < 0.001$) and vertebral injury ($P < 0.001$). Hemorrhages were about 9 (95% CI 3.6 – 22) times more likely to occur in fish that were immobilized compared to those demonstrating an escape response. Those fish exhibiting forced swimming were about 6 (95% CI 2.4 – 17) times more likely to demonstrate hemorrhaging than those reacting with the escape response. Immobilized fish were 4.2 (95% CI 2.0 – 9.0) times more likely to have vertebral injury than those demonstrating an escape response. Those fish that reacted with forced swimming were at a slightly higher risk for vertebral injury than those reacting with an escape response (OR = 2.6; 95% CI 1.1 – 6.2). Model (R) had an acceptable level of discriminatory capabilities for hemorrhage (area under the ROC curve = 0.74), but not for vertebral injury, as indicated by the area under the ROC curve (0.66).

Largemouth bass.—A total of 400 pond-reared largemouth bass in two size groups (corresponding to age 0 and ages 1, 2, and 3) were exposed to DC, 30-Hz PDC, or 60-Hz PDC at 0.1, 0.2, or 0.5 V/cm, or used as controls (Table 2). The electrical treatments were applied to the test tank at 15 V (± 1.1 V), 30 (±

1.4 V), or 60 V (± 1.1 V). Water temperature in the test tank was between 21 and 23°C (21.9 ± 0.7 C). The ambient conductivity of the water in the test tank ranged between 528-583 μS/cm (556 ± 20 μS/cm).

Largemouth bass in the small size group (age 0) were 103-158 mm (131 mm ± 7) total length and weighed 11-42 g (23 g ± 4). Fish in the large size group (ages 1, 2, and 3) were 218-376 mm (269 mm ± 29) total length and weighed 121-572 g (248 g ± 78). Mean length ($P = 0.0001$) and mean weight ($P = 0.0001$) differed significantly between the two size groups, as indicated by the two sample t-tests.

Hemorrhage Evaluation.— Overall, hemorrhage was detected in 6% (21/360) of the largemouth bass exposed to electrical treatment. No hemorrhages were detected in fish designated as controls from either size group. There was no statistically significant difference in hemorrhage rate between the large and small size groups of largemouth bass, when pooled across waveforms and voltage gradients (11/180 (6%) versus 10/180 (6%); $P = 1.000$). When pooled across size group and voltage gradient, hemorrhage rate differed significantly among the three electrical waveforms: 15/120 (13%) for 60-Hz PDC; 3/120 (3%) for 30-Hz PDC; and 3/120 (3%) for DC ($P = 0.001$). When pooled across waveforms and size groups, there was a significant correlation between hemorrhage rate and voltage gradient: 17/120 (14%) for 0.5 V/cm; 4/120 (3%) for 0.2 V/cm; and 0/120 for 0.1 V/cm ($P = 0.001$).

When stratified by waveform, the significant correlation between voltage gradient and hemorrhage rate persisted (P = 0.001). Controlling for 60-Hz PDC, a significant trend between voltage gradient and hemorrhage rate was demonstrated: 13/40 (33%) for 0.5 V/cm; 2/40 (5%) for 0.2 V/cm; and 0 /40 (0%) for 0.1 V/cm (P = 0.000). Conversely, no significant differences in hemorrhage rate were demonstrated for the voltage gradients, when controlling for 30-Hz PDC: 1/40 (3%) for 0.5 V/cm; 2/40 (5%) for 0.2 V/cm; and 0/40 (0%) for 0.1 V/cm (P = 0.737). No statistically significant trend between voltage gradient and hemorrhage rate was evident, when controlling for DC. However, hemorrhage rate was higher for fish exposed to 0.5 V/cm than for those exposed to 0.2 or 0.1 V/cm: 3/40 (8%) for 0.5 V/cm, 0/40 for 0.2 V/cm and 0.1 V/cm (P = 0.070).

When stratified by size group and electrical waveform, a significant correlation between voltage gradient and hemorrhage rate was demonstrated (P = 0.001; Table 17). Though a trend between voltage gradient and hemorrhage rate was demonstrated in the large size group exposed to 60-Hz PDC, the trend was not statistically significant: 4/20 (20%) for 0.5 V/cm; 2/20 (10%) for 0.2 V/cm; and 0/20 for 0.1 V/cm (P = 0.063). No trend was evident between voltage gradient and hemorrhage rate in the large size group of largemouth bass exposed to 30-Hz PDC: 1/20 (5%) for 0.5 V/cm; 1/20 (5%) for 0.2 V/cm; and 0/20 (0%) for 0.1 V/cm (P = 0.667). Nor was a statistically significant trend between voltage gradient and hemorrhage rate evident in the large size group exposed to continuous DC: 3/20 (15%) for 0.5 V/cm; 0/20 for 0.2 and 0.1 V/cm (P = 0.067).

Table 17.— Largemouth bass hemorrhage rate (trends). Tests for trend between voltage gradient and hemorrhage rate in largemouth bass, when controlling for size group and electrical waveform.

Fish size	Waveform	Hemorrhage Rate (0.5 V/cm vs. 0.2 V/cm vs. 0.1 V/cm)	P-value
Large	60-Hz PDC	4/20 vs. 2/20 vs. 0/20	0.063
Large	30-Hz PDC	1/20 vs. 1/20 vs. 0/20	0.667
Large	DC	3/20 vs. 0/20 vs. 0/20	0.067
Small	60-Hz PDC	9/20 vs. 0/20 vs. 0/20	0.000
Small	30-Hz PDC	0/20 vs. 1/20 vs. 0/20	1.000
Small	DC	0/20 vs. 0/20 vs. 0/20	—

A significant trend between hemorrhage rate and voltage gradient was demonstrated in the small size group exposed to 60-Hz PDC: 9/20 (45%) for 0.5 V/cm; 0/20 for 0.2 and 0.1 V/cm (P < 0.001). No statistically significant trends between voltage gradient and hemorrhage rate were demonstrated for the remaining groups of small largemouth bass exposed to DC pulsed at 15-Hz or DC (P = 1.000; Table 17).

Vertebral Injury Evaluation.—Radiographic images of diagnostic quality were obtained for 90% (18/20) of the bass designated as controls in the small size group and 100% of those large size group. No vertebral injury was detected in any fish from either size group of control fish. Radiographs of diagnostic quality were obtained for 94% (339/360) of the largemouth bass exposed to electrical treatments: 180/180 (100%) of the large size group; 159/180 (88%) of the small size group of fish.

Overall, vertebral injury was detected in 7% (24/339) of the largemouth bass exposed to electrical treatment (Table 18). Vertebral injury rate was greater in the large size group, compared to the small size group [18/180 (10%) versus 6/159 (4%); RR =2.7; P = 0.033], when pooled across waveforms and voltage gradients. Vertebral injury rate varied significantly among the three electrical waveforms, when pooled across size groups and voltage gradients: 16/110 (15%) for 60-Hz PDC; 5/116 (4%) for 30-Hz PDC; and 3/113 (3%) for DC. There was evidence for a significant correlation between voltage gradient and vertebral injury rate, when vertebral injury rate was pooled across size groups and

Table 18.— Largemouth bass vertebral injury rate (trends). Tests for trend between voltage gradient and vertebral injury rate in largemouth bass, when controlling for size group and electrical waveform.

Fish size	Waveform	Hemmorhage rate (0.5 V/cm vs. 0.2 V/cm vs. 0.1 V/cm)	P-value
Large	60-Hz PDC	4/20 vs. 4/20 vs. 2/20	0.531
Large	30-Hz	1/20 vs. 2/20 vs. 2/20	0.781
Large	DC	3/17 vs. 0/20 vs. 0/20	0.067
Small	60-Hz PDC	6/15 vs. 0/17 vs. 0.18	< 0.001
Small	30-Hz	0/20 vs. 0/16 vs. 0/20	—
Small	DC	0/17 vs. 0/17 vs. 0/19	—

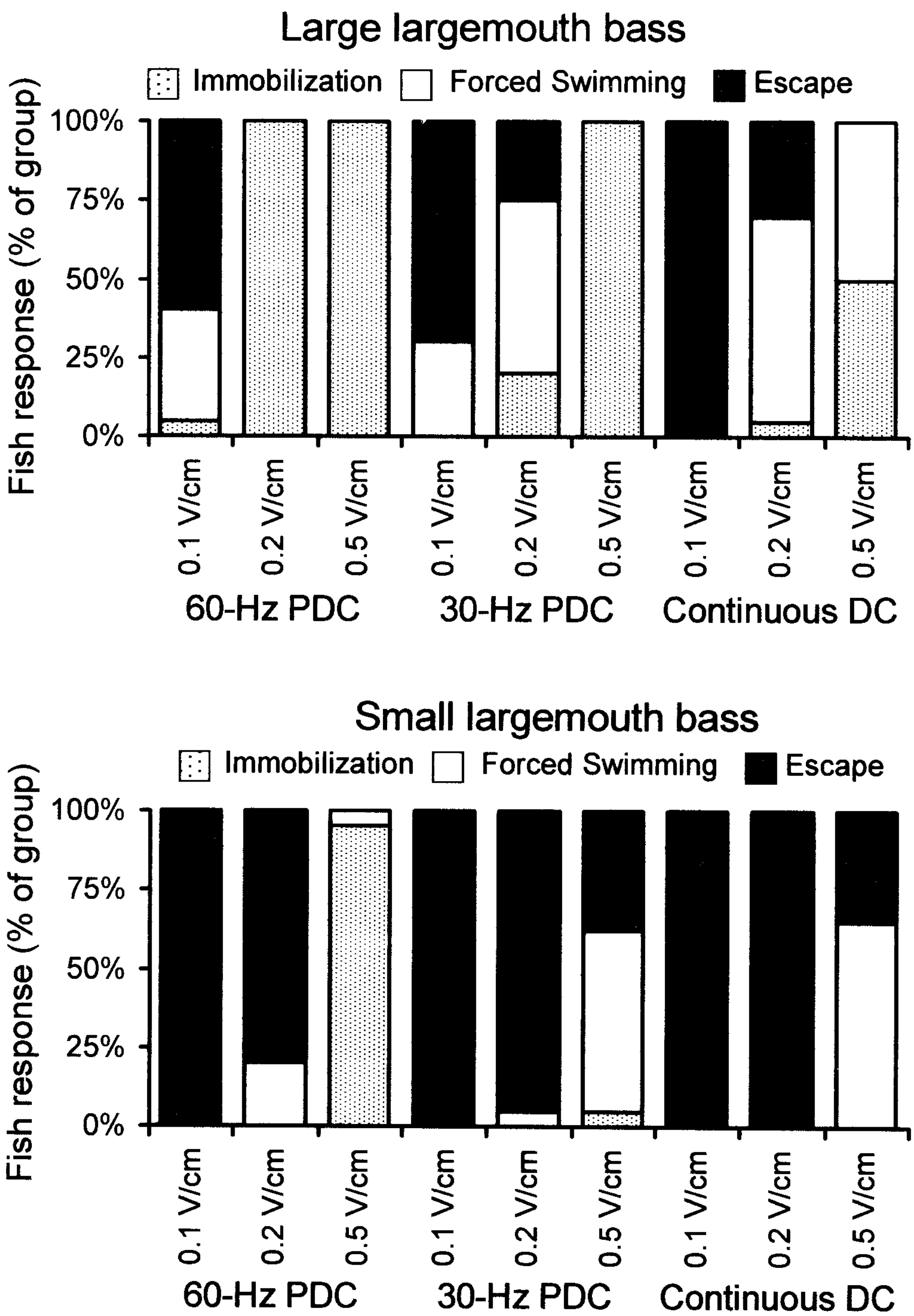


Figure 5. — Largemouth bass behavioral responses. Behavioral responses induced in largemouth bass during exposure to various electrical treatments

electrical waveforms: 14/112 (13%) for 0.5 V/cm; 6/110 (6%) for 0.2 V/cm; and 4/117 (3%) for 0.1 V/cm ($P = 0.008$). Significant statistical evidence was presented for a trend in vertebral injury rate and voltage gradient for small largemouth bass that were exposed to 60-Hz PDC ($P < 0.000$). No statistically significant trends for vertebral injury rate to vary with voltage gradient was noted for the remaining experimental groups (Table 18).

Induced Behavior and Injury.—Of the 360 largemouth bass exposed to electrical treatment, 27% were immobilized, 22% demonstrated forced swimming behaviors, and 52% responded with escape reactions (Figure 5). Hemorrhage rate varied significantly among the behavioral responses of large largemouth bass: 12% of those immobilized; 4% of those demonstrating forced swimming; 0% of those with escape responses ($P = 0.016$). A statistically significant trend for hemorrhage rate to decrease with perceived severity of behavioral response of large bass was evident ($P = 0.016$). Although there was no statistically significant difference in large bass hemorrhage rate between fish demonstrating forced swimming behaviors and those immobilized ($P = 0.202$) or those exhibiting escape reactions ($P = 0.202$), risk for hemorrhage was significantly higher for immobilized bass than for those reacting with escape responses ($P = 0.001$; Table 19). Hemorrhage rate differed significantly among the behavioral responses of the small bass: 45% for immobilization; 0% for forced swimming; and 0% for the escape response ($P = 0.001$). In addition, a significant trend for hemorrhage rate to decrease with perceived severity of behavioral response was

Table 19.— Largemouth bass behavioral response and injury. Rate of hemorrhage among largemouth bass exposed to various electrical treatments. Relative risk (RR) and (in parentheses) 95% confidence limits, with associated P-values are shown for each comparison.

Group comparisons	Hemorrhage rate	RR	P-value
Large Fish			
Escape vs. immobilization	0/57 vs. 9/76	0.1 (0.04-0.2)	0.001
Escape vs. forced swimming	0/57 vs. 2/47	0.2 (0.07-0.8)	0.202
Forced swimming vs. immobilization	2/47 vs. 9/76	0.4 (0.07-1.2)	0.202
Small Fish			
Escape vs. immobilization	1/129 vs. 9/20	0.02 (0.0-0.1)	< 0.001
Escape vs. forced swimming	1/129 vs. 0/31	0.73 (0.3-1.7)	1.000
Forced swimming vs. immobilization	0/31 vs. 9/20	0.03 (0.0-0.1)	< 0.001

detected (P < 0.001). Although vertebral injury rate did not significantly differ among the responses of large sized bass to electrical treatment (P = 0.061), a significant trend was evident: 16% for immobilization; 9% for forced swimming; and 4% for escape responses. Vertebral injury rate varied significantly among small bass responses to electrical treatment (P = 0.001) and a significant trend was detected: 40% for immobilization; 0% for forced swimming; and 0% for escape responses.

Injury Model Selection and Evaluation.—Among the 360 largemouth bass examined for injury, hemorrhage was detected in 6% of the fish. No largemouth bass exposed to the lowest voltage gradient suffered hemorrhage, resulting in a quasi-complete separation in the data that was accompanied by poor parameterestimation and large standard errors resulted in the voltage gradient model. Evaluation of the candidate set of models continued after elimination of the lowest voltage gradient category. Hemorrhage was detected in 9% of the fish of the remaining 240 largemouth bass evaluated for internal hemorrhage. Univariate analysis demonstrated voltage gradient (P = 0.006) and electrical waveform (P = 0.0020) were statistically significant single predictors of largemouth bass hemorrhage rate; fish size was not (P = 0.819). The waveform and voltage gradient instrument (W, E) was the single best model for describing hemorrhage rate in electroshocked largemouth bass, as indicated by the model selection criteria (Table 20). Comparison of the Akaike weights of the (W, E) and the next best model, the (W, E, S) model, indicated the (W, E) model

Table 20.—Largemouth bass model selection (hemorrhage). Summary of selection statistics for models relating the experimental variables to the occurrence of hemorrhage in largemouth bass. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Model	Δ_i	ω_i	ROC	GOF
(W, E)	0.0	0.71	0.77	0.42
(W, E, S)	1.9	0.27	0.79	0.14
(W)	8.1	0.01	0.71	—
(W, S)	10.0	0.00	0.73	0.18
(E)	10.7	0.00	0.67	—
(S, E)	12.6	0.00	0.68	0.59
(S)	20.0	0.00	0.52	—

$\left(\omega_{(W,E)} = 0.71\right)$ was about 2.5 times as likely to be the best model for hemorrhage rate in largemouth bass, as the (W, E, S) model $\left(\omega_{(W,E,S)} = 0.27\right)$. Electrical waveform (P = 0.002) and voltage gradient (P = 0.005) effects were predictive of hemorrhage in largemouth bass. Bass exposed to 60-Hz PDC were more likely to suffer hemorrhage than those exposed to DC (OR = 6.4, 95% CI 1.7-29.7; P = 0.005) or 30-Hz PDC. There was no statistically significant difference in hemorrhage rate between largemouth bass exposed to 30-Hz PDC and those exposed to DC (OR = 1.0, 95% CI 0.2-5.2; P = 1.000). Those largemouth bass exposed to 0.5 V/cm were more likely to suffer hemorrhage than those exposed to 0.2 V/cm (OR = 5.3, 95% CI 1.7-15.9; P = 0.005). The area under the ROC curve (ROC = 0.77) indicated the instrument to have acceptable discriminatory ability.

Among the 339 largemouth bass evaluated with radiographs, 7% had vertebral injury. Univariate analysis demonstrated that fish size (S; P = 0.032), electrical waveform (W; P = 0.003), and voltage gradient (E; P = 0.029) were each statistically significant single predictors of vertebral injury rate in largemouth bass. Of the three univariate models relating largemouth bass size (S), electrical waveform (W), and voltage gradient (E) to vertebral injury rate, the electrical waveform model (W) was the only model with an acceptable level of discrimination of outcome, as indicated by the area under the ROC. The electrical waveform model had an area under the ROC of 0.70 (at the lower

Table 21.— Largemouth bass model selection (vertebral injury). Summary of selection statistics for models relating the experimental variables to vertebral injury in largemouth bass. The area under the receiver-operating characteristic curve is designated by ROC and the Hosmer-Lemeshow goodness-of-fit test P-value is designated GOF.

Models	Δ_i	ω_i	ROC	*GOF*
(W, E, S)	0	0.72	0.82	0.47
(W, E)	3	0.16	0.75	0.65
(W, S)	4	0.09	0.76	0.38
(W)	7	0.02	0.70	1.00
(S, E)	10	0.01	0.71	0.21
(E)	13	0.00	0.66	1.00
(S)	13	0.00	0.62	—

margin of the acceptable interval, the largemouth bass size model an area of 0.618, and the voltage gradient model an area of 0.66.

Comparison of AIC values for the models within the candidate set indicated the (W, E, S) model as the best. However, the Δ values also demonstrated some support for the (W, E), (W, S) and (W) models. Comparison of model Akaike weights demonstrated the (W, E, S) model as the single best model: the (W, E, S) model was 4.5 times more likely than the (W, E) model, eight times more likely than the (W, S) model, and 36 times more likely than the (W) model (Table 21). Significant waveform (P = 0.002), voltage gradient (P = 0.0219), and fish size (P = 0.034) effects were demonstrated in the (W, E, S) model. Risk of vertebral injury was significantly greater in largemouth bass exposed to 60-Hz PDC than for those exposed to DC (OR = 6.7, 95% CI 2.1-30; P = 0.004) or 30-Hz PDC (P = 0.011). There was no statistically significant difference in risk for vertebral injury between largemouth bass exposed to 30-Hz PDC and those exposed to DC (OR = 1.675, 95% CI 0.4-8.4; P = 0.492). There was no statistically significant difference in risk of vertebral injury between largemouth bass exposed to 0.5 V/cm and those exposed to 0.2 V/cm (P = 0.055). There was no statistically significant difference in risk between fish exposed to 0.2 V/cm and those exposed to 0.1 V/cm (OR = 1.5, 95% CI 0.4- 6.5; P = 0.4874). However, fish exposed to 0.5 V/cm had a significantly greater risk of vertebral injury than those exposed to 0.1 V/cm (OR = 4.4, 95% CI 1.5-16; P = 0.0137). The (W, E, S) model had very good discriminatory capacity, as indicated by the area under the ROC of 0.82. Further, the Hosmer-Lemeshow goodness-of-fit test indicated that the model described the data adequately ($P = 0.47$).

The models relating evoked response to injury indicated fish response to be independently predictive of hemorrhage ($P < 0.001$) and vertebral injury ($P < 0.001$). Hemorrhages were about 43 (95% CI 8.6 – 775) times more likely to occur in fish that were immobilized compared to those demonstrating an escape response. Immobilized fish were about 21 (95% CI 5.8 – 134) times more likely to have vertebral injury than those demonstrating an escape response. Those fish exhibiting forced swimming or escape were at similar risk for hemorrhage (OR = 4.9; 95% CI 0.5 – 105) or vertebral injury (OR = 4.7; 95% CI 0.9 – 35). Model (R) had a good discriminatory capabilities for hemorrhaging (area under the ROC curve = 0.83) and vertebral injury (0.80).

Bluegill.—A total of 400 pond-reared bluegill sunfish in two size groups, designated as large and small, were either exposed to DC, 30 Hz PDC, or 60-Hz PDC at 0.2, 0.5, 0.6 V/cm, or were used as controls (Table 2). The electrical treatments were applied to the test tank electrodes at voltages of 30 V (SD; ± 1.2 V), 60 V (± 2.8 V), or 80 V (± 1.3 V). Water conditions varied little over the course of the experiment; temperature ranged between 20-23°C (21.3 ± 1.3°C) and ambient conductivity between 534-581 (557 ± 16 μS/cm).

The bluegill in the small size group (age 0) were 60-103 mm (75 mm ± 7) total length and weighed 3-19 g (6 g ± 2). Fish in the large size group (ages 3 and 4) were 150-226 mm (186 mm ± 13) total length and weighed 65-198 g (116 g ± 23). Mean length (P = 0.0001) and mean weight (P = 0.0001) differed significantly between the two size groups, as indicated by the two sample ttests.

Injury Evaluation.—Overall, hemorrhage was detected in two of the 360 bluegill exposed to electroshock. The hemorrhages, which occurred in large bluebill exposed to 60-Hz PDC, were categorized as class 2. One fish had been exposed to 0.5 V/cm, the other to 0.6 V/cm. Vertebral injury was detected in one of the 360 bluebill exposed to electroshock. The single vertebral injury occurred the large bluegill that was exposed to 60-Hz PDC at 0.6 V/cm that had a hemorrhage.

Induced Behavior and Injury.— Overall, evoked responses favorable for capture during electrofishing (immobilization or forced swimming) were evoked from 92% of the large bluegill exposed to electroshock and 59% of the small bluegill (Figure 6). The immobilization response was evoked from 95% of the large bluegill exposed to 60-Hz PDC, 68% of those exposed to 30-Hz PDC, and 23% of those exposed to DC. Forced swimming behaviors were evoked from 62% of the large bluegill exposed to DC. Both large bluegills with hemorrhages, including the single vertebral injury, had been immobilized during electrical treatment.

Injury Model Selection and Evaluation.— Injury, internal hemorrhage associated with the vertebral column or vertebral injury, was detected in less than 1% of the 360 bluegill exposed to electrical treatment. The null model predicts that the probability of electroshock-induced injury is constant. The probability of

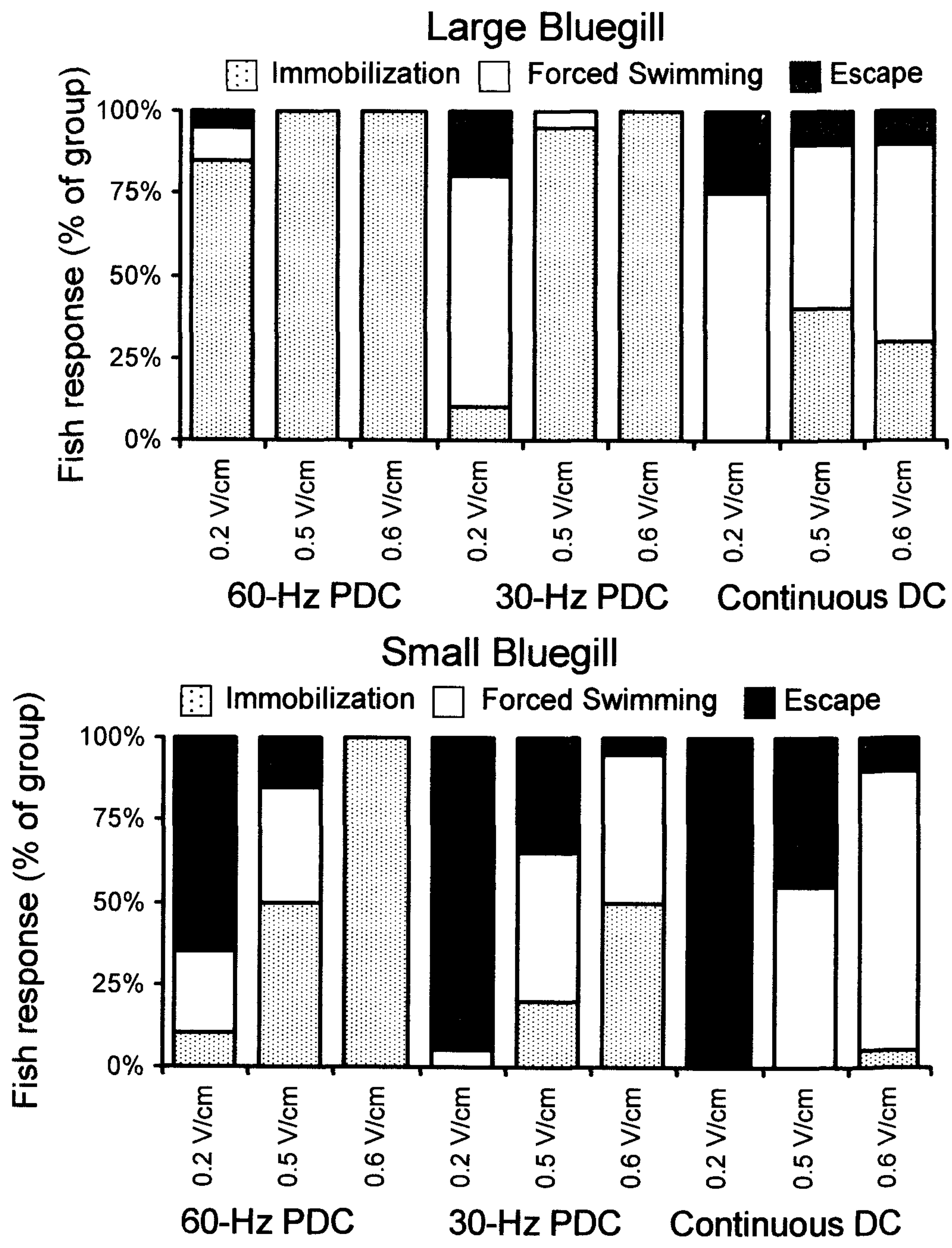


Figure 6. — Bluegill behavioral responses. Behavioral responses induced in bluegill duringexposure to various electrical treatments.

electroshock-induced injury in bluegill was very low. No risk factors for injury were identified for bluegill.

Hybrid striped bass.—A total of 400 hybrid striped bass in two size groups, designated large and small, were either exposed to continuous DC, 30 Hz PDC, or 60 Hz PDC at 0.2, 0.5, or 0.6 V/cm, or were used as controls. Treatments were applied to the test tank at voltages of 30 V (± 1.2 V), 60 V (± 3 V), or 80 V (± 1.3 V). Water conditions in the test tank varied little over the course of the experiment: water temperature ranged between 19-20°C (19 ± 0.3) and ambient conductivity between 787-855 μS/cm (821 ± 21 μS/cm).

The striped bass hybrids in the small size group were 172-243 mm (204 mm ± 10) total length and weighed 58-127 g (93 g ± 13). Fish in the large size group were 323-421 mm (379 mm ± 18) total length and weighed 427-989 g (716 g ± 106). Mean length (P = 0.0001) and mean weight (P = 0.0001) differed significantly between the two size groups, as indicated by the two sample t-tests.

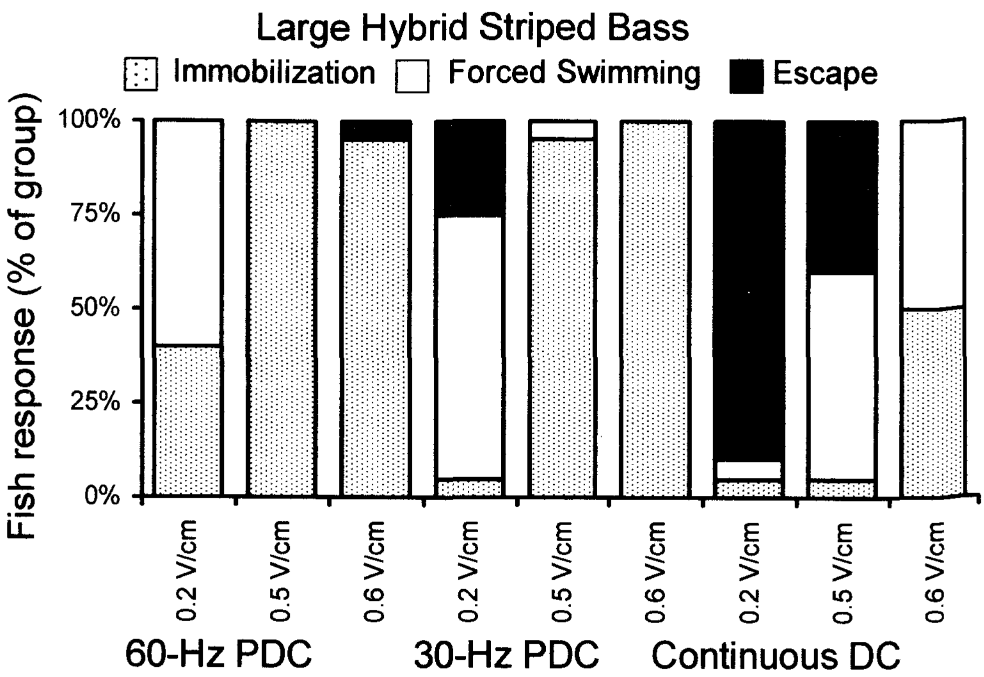
Hemorrhage Evaluation.—Injury was evaluated in 399 hybrid striped bass via bilateral filleting. Data for one large fish was mistakenly not recorded. No injury was detected in control fish from either size group. Hemorrhage along the vertebral column was detected in 2% (6/359) of the hybrid striped bass exposed to electrical treatment. All of the hemorrhages, which were categorized as class 2 in perceived severity occurred in large hybrid striped bass. No hemorrhages were detected in small hybrid striped bass.

Hemorrhage rate was significantly greater in large hybrid striped bass [6/179 (3%)] compared to small bass (0/180 (0%); P = 0.015). Hemorrhage rate in large hybrid striped bass varied significantly among the three waveforms, when pooled across the voltage gradients: 5/60 (8%) for 60-Hz PDC; 1/60 (2%) for 30-Hz PDC; and 0/60 (3%) for DC (P = 0.028). However, pair-wise comparisons of hemorrhage rates between the waveforms failed to detect statistically significant differences: 60-Hz PDC versus 30-Hz PDC (P = 0.207); 60-Hz PDC versus DC (P = 0.057); 30-Hz PDC versus DC (P = 1.000). No statistically significant difference in hemorrhage rate of large hybrid striped bass was noted among the three voltage gradients, when pooled across waveform: 3/59 (5%) for 0.6 v/cm; 2/60 (3%) for 0.5 V/cm; and 1/60 (2%) for 0.2 V/cm (P = 0.587). No statistically significant trend in hemorrhage rate was associated with voltage gradient evident for large fish (P = 0.300).

Vertebral Injury Evaluation.—Radiographs of diagnostic quality were obtained for all the fish used in the study. No vertebral injury was detected in fish designated as controls. Overall, vertebral injury was detected in 2% (7/360) of the hybrid striped bass exposed to electroshock. Vertebral injury rate was greater in large hybrid striped bass [6/180 (3%)] compared to small hybrid striped bass [1/180 (1%)], the difference, however, was not statistically significant (P = 0.121). Vertebral injury rate varied significantly among the three electrical waveforms, when pooled across fish size and voltage gradient: 6/120 (5%) for DC 60-Hz PDC; 0/120 for 30-Hz PDC; and 1/120 (1%) for DC (P = 0.011). No statistically significant voltage gradient effects were demonstrated when vertebral injury rate was pooled across fish size and electrical waveform: 4/120 (3%) for 0.6 V/cm; 1/120 (1%) for 0.5 V/cm; and 2/120 (2%) for 0.2 V/cm (P = 0.362). There was no evidence for a trend in vertebral injury associated with voltage gradient, when pooled across fish size and waveform (P = 0.350).

Concurrent Hemorrhage and Vertebral Injury.—Overall, injury, hemorrhage or vertebral injury, was detected in 3% (11/359) of the hybrid striped bass exposed to electroshock. Injury occurred more often in large fish [10/179 (6%)] than in small hybrid striped bass [1/180 (1%)]. Two fish (18%) had injuries detected by both methods; vertebral injury was detected in 33% (2/6) of the fish with a positive hemorrhage status, hemorrhage was detected in 28% (2/7) of the fish with positive vertebral injury status.

Induced Behavior and Injury.—Of the 360 hybrid striped bass exposed to electroshock 47% were immobilized, 28% exhibited forced swimming, and 26% demonstrated escape responses to electrical treatment (Figure 7). Thus, behaviors favorable for capture during electrofishing were evoked from 75% of the hybrid striped bass in the study. No statistically significant difference in hemorrhage rate was noted among the evoked responses: 5/168 (3%) for the immobilization response; 1/99 (1%) for forced swimming behaviors; and 0/92 (0%) for escape reactions (P = 0.169). Though hemorrhage rate decreased with severity of behavioral response, no statistically significant trend was evident (0.062). Likewise, no statistically significant differences in vertebral injury rates



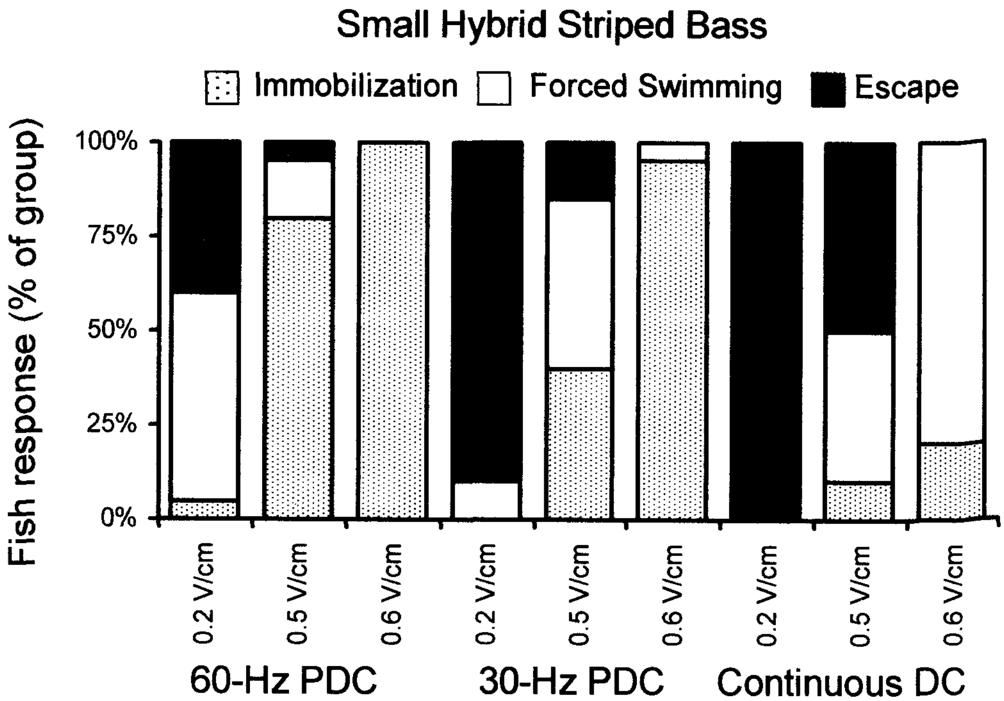


Figure 7. — Hybrid striped bass behavioral responses. Behavioral responses induced in hybrid stripedbass during exposure to various electrical treatments.

were noted among the behavioral responses: 6/168 (4%) for immobilized fish; 1/99 (1%) for those demonstrated forced swimming; and 0/92 for those exhibiting escape responses during treatment (P = 0.103). No statistically significant trend in vertebral injury rate was associated with behavioral response (P = 0.037).

Injury Model Selection and Evaluation.—Of the 360 hybrid striped bass exposed to electrical treatment, hemorrhage was detected in 2%. No hemorrhages were detected in small bass, thus data for small bass were eliminated during model selection procedures. Because no hemorrhages were detected in bass exposed to DC this category was also eliminated from the analysis. Hemorrhage was detected in 5% of the remaining 120 large sized hybrid striped bass.

Univariate analysis demonstrated that neither electrical waveform (P = 0.1307) nor voltage gradient (P = 0.6131) were singly predictive of hemorrhage outcome in hybrid striped bass. Similarly, waveform (P = 0.1288) and voltage gradient (P = 0.6057) did not have statistically significant effects in the (W, E) model. The (W) model had the lowest AIC value of the three models, followed by the (W, E) model ($\Delta = 3$) and the (E) model ($\Delta = 4$). The model Akaike weights indicated that the (W) model was about four times more likely than the (W, E) model and about seven times more likely than the (E) model, to be the best model. Additionally, fish response was not independently predictive of hemorrhage (P = 0.393) or vertebral injury (P = 0.398).

RESULTS OF GENERAL MODELS FOR INJURY

The data from the electroshock-induced injury experiments were combined, creating a single data set for exploration of a prognostic model for elecroshock-induced injury, including a mechanistic underpinning. A total of 2012 fish, including chinook salmon, rainbow trout, channel catfish, largemouth bass, bluegill, and hybrid striped bass, that had been exposed to electrical treatment, comprised the data set. The pooling of the data sets introduced a new variable for analysis, fish species (Sp).

Review of individual predictors of injury in the individual experiments demonstrated the fish size (S) and fish response (R) were the most consistently statistically significant variables among those evaluated (Table 22). In comparison, the variables describing electrical stimulus (waveform and voltage gradient) did not perform as well. Because the electrical waveforms and voltage gradients were not replicated throughout the experiments, these variables were dropped from further analysis in favor of using fish response, which was an observable manifestation of *in vivo* electrical energy.

Prognostic models relating fish size group (S), fish response (R), and species (Sp) to the occurrence of hemorrhage and vertebral injury were evaluated. Further, the predictive capacity of fish length (L) and fish mass (weight; W) as continuous variables, were evaluated. However, because the

Table 22.—Predictors of fish injury. Summary of predictors of fish injury in the series of experiments evaluating the effects of electroshock. Statistically significant single predictors ($P < 0.05$) of hemorrhage (H) and vertebral injury (V) for each fish species are indicated.

Fish species	Waveform (W)	Voltage gradient (E)	Size group (S)	Fish response (R)
Chinook salmon	H	H	H, V	H
Rainbow trout	H, V		H, V	H, V
Channel catfish	H, V	H	H, V	H, V
Largemouth bass	H, V	H, V		H
Bluegill				
Hybrid striped bass			H, V	H, V

species were not evenly distributed according to size, the data set was divided into small (≤ 230 mm) and large size groups (> 230 mm; Figure 8) and further analysis performed on these size groups. All the species were represented in the small size group. The large size group was comprised of largemouth bass, hybrid striped bass, channel catfish, and rainbow trout.

Models for Hemorrhage.— Univariate analysis of fish length indicated length was singly predictive of hemorrhage rate ($P < 0.0001$). The (L) model had an area under the ROC curve of 0.70, indicating a level of predictive discrimination that falls, barely, within the interval of acceptable. An odds ratio of 1.007 was predicted for fish length, indicated that the odds of hemorrhage increase by 1.007 for each 1 mm increase in length. The Hosmer-Lemeshow GOF test rejected the null hypothesis that the model fit the data ($P < 0.0001$).

Univariate analysis indicated fish weight (W) to be singly predictive of hemorrhage rate ($P < 0.0001$). The model estimated that the likelihood of hemorrhage increased 1.002 (95% CI 1.001-1.002) for every 1 g increase in mass. The (M) model had an area under the ROC curve of 0.68, indicating unacceptable predictive discriminatory capacity. The Hosmer-Lemeshow GOF test rejected the null hypothesis that the model fit the data ($P < 0.0001$).

Univariate analysis demonstrated fish species (Sp) to be independently predictive of hemorrhage rate ($P < 0.0001$). The (Sp) model indicated chinook salmon and rainbow trout were at similar risk of injury (OR = 1.176, 95% CI

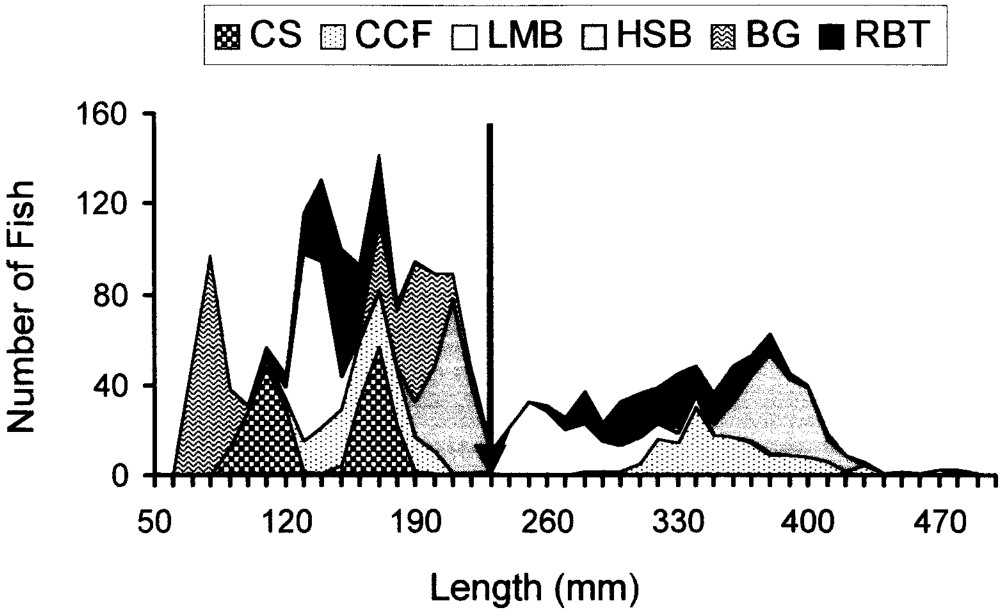


Figure 8.—Fish lengths in the pooled data set. Plot of numbers of fish as a function of fish length for the various species in the data set. The arrow indicates the division of fish length into groups ≤ 230 mm and > 230 mm.

0.708-1.952). Hemorrhage rate was significantly higher in channel catfish compared to rainbow trout (OR = 1.130-2.706). Largemouth bass were almost two times less likely to suffer hemorrhage compared to rainbow trout (OR = 0.510, 95% CI 0.294-0.886). Hybrid striped bass were almost seven times less likely to have hemorrhages than rainbow trout (OR = 0.140, 95% CI 0.058-0.335). Bluegill were almost 22 times less likely to suffer hemorrhage than rainbow trout (OR = 0.046, 95% CI 0.011-0.192). The (species) model had an area under the ROC curve of 0.74, indicating acceptable predictive discrimination. The Hosmer-Lemeshow GOF test failed to reject the null hypothesis that the model fit the data (P = 1.0000).

Univariate analysis indicated induced fish response was singly predictive of hemorrhage rate (P < 0.0001). Hemorrhage rate was significantly more likely when the immobilization (OR = 9.2, 95% CI 5.5-16.5) or forced swimming (OR = 2.7, 95% CI 1.4-5.3) was induced compared when fish reacted with an escape response. The (R) model had an acceptable level of predictive discrimination, as indicated by an area under the ROC curve of 0.72). The GOF test failed to the reject the null hypothesis that the (R) model fit the data (P = 0.9998

Univariate analysis indicated size group as a significant single predictor of hemorrhage rate (P < 0.0001). Further analysis was conducted on each size group of fish. Hemorrhage was about five times more likely in fish greater than 230 mm compared to those fish 230 mm or less in length (OR = 4.8, 95% CI 3.4-6.8). However, the model had poor predictive discrimination (ROC = 0.69).

Induced response was a significant single predictor of hemorrhage rate in those fish less than 230 mm in length (P < 0.0001). Hemorrhage rate was about 11 times greater in immobilized small fish compared to those exhibiting the escape response (OR = 10.8, 95% CI 4.6-31.7). Hemorrhage rate was similar for small fish exhibiting forced swimming responses and escape (OR = 2.1, 95% CI 0.6-7.8). The (R) model had an acceptable level of predictive discrimination (ROC = 0.75). The GOF test failed to reject the null hypothesis that the model fit the data (P = 0.9998).

Evaluation of species as a predictor of hemorrhages in small fish demonstrated a quasi-complete separation in the data accompanied by poor estimation of model coefficients. Removal of hybrid striped bass from the data set corrected the problem, because no hemorrhages had been detected in any small hybrid striped bass. Species was a significant single predictor of hemorrhage rate in the remaining four species (P < 0.0001). Model (Sp) indicated risk for hemorrhage was considerably less in small channel catfish (OR = 0.131, 95% CI 0.021-0.445), largemouth bass (OR = 0.494, 95% CI 0.237-0.971), and bluegill (OR = 0.039, 95% CI 0.006-0.131) compared to chinook salmon. The model had acceptable predictive discrimination (ROC = 0.77). The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Species was a significant single predictor of hemorrhage rate in the large fish (P < 0.0001). Model (Sp) indicated hemorrhage rate was significantly higher in channel catfish (OR = 2.0, 95% CI 1.2-3.2) compared to rainbow trout. Hemorrhage rate was significantly less in largemouth bass (OR = 0.2, 95% CI 0.1-4) and hybrid striped bass (OR = 0.1, 95% CI 0.05-0.3) compared to rainbow trout (Figure 9). The model had an acceptable level of predictive discrimination (ROC = 0.75). The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Models for Vertebral Injury.— Univariate analysis of fish length (L) indicated the variable was independently predictive of vertebral injury rate (P < 0.0001). The odds of vertebral injury were predicted to increase by 1.008 for each 1 mm increase in fish length. The (L) model had an area under the ROC curve of 0.73, indicating an acceptable level of predictive discrimination. The Hosmer-Lemeshow GOF test rejected the null hypothesis that the model fit the data (P < 0.0001).

Univariate analysis demonstrated fish weight (W) to be independently predictive of vertebral injury rate (P < 0.0001). The likelihood of vertebral injury increased 1.002 (95% CI 1.001-1.002) for each 1g increase in mass. The (W) model had an area under the ROC curve of 0.71, indicating an acceptable level of predictive discrimination. The Hosmer-Lemeshow GOF test rejected the null hypothesis that the model fit the data (P < 0.0001).

Univariate analysis demonstrates species (Sp) to be independently predictive of vertebral injury rate (P < 0.0001). The (Sp) model predicted that vertebral injury was less likely to be detected in chinook salmon (OR = 0.552, 95% CI 0.314-0.971), largemouth bass (OR = 0.489, 95% CI 0.291-0.823), hybrid striped bass (OR = 0.127, 95% CI 0.057-0.287), and bluegill (OR = 0.018, 95% CI 0.002-0.131) compared to rainbow trout. Channel catfish and rainbow trout were at similar risk of injury (OR = 1.295, 95% CI 0.831-2.019). This model had an area under the ROC curve of 0.74, indicating an acceptable level of predictive discrimination. The Hosmer-Lemeshow GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Fish response (R), as a univariate, was independently predictive of vertebral injury rate (P < 0.0001). Vertebral injury rate was significantly greater in fish that had been immobilized (OR = 4.9, 95% CI 3.1-81) compared to those exhibiting an escape response to electroshock. Whereas, vertebral injury rate was similar when the forced swimming response was induced compared to the escape response (OR = 1.6, 95% CI 0.9-3.0). The (R) model had poor predictive discriminatory capacity, as indicated by an area under the ROC curve of 0.68. The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Fish size was demonstrated to be an independent predictor of vertebral injury rate in univariate analysis on the two size groups (P < 0.0001). Vertebral injury was more likely in large fish compared to small fish (OR = 7, 95% CI 4.2-9.1). The model had a level of predictive discrimination at the lower margin of acceptability (ROC = 0.71).

Induced behavioral response was indicated to be a significant single predictor of vertebral injury rate in the small fish (P = 0.0002). Vertebral injury rate was about 4 times greater in immobilized fish compared to those demonstrating an escape response (OR = 4.2, 95% CI 2.0-10.1). Vertebral injury rate was similar in fish showing forced swimming compared to escape responses (OR = 0.75, 95% CI 0.2-2.9). The model had poor predictive discrimination (ROC = 0.69). The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Fish species was a significant single predictor of vertebral injury rate in small fish (P = 0.0006). Using bluegill as a baseline, vertebral injury rate was significantly greater (OR = 31, 6.3-556) in chinook salmon and largemouth bass (OR = 18.7, 95% CI 3.4-350). Vertebral injury rate in small channel catfish was similar to that of chinook salmon (OR = 4.5, 95% CI 0.2-114). Model (Sp) had acceptable predictive discrimination (ROC = 0.79). The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

Species was a significant single predictor of vertebral injury rate in large fish (P < 0.0001). Vertebral injury rate was similar between channel catfish and rainbow trout (OR = 1.4, 95% CI 0.8-2.2). However, vertebral injury rate was less in largemouth bass (OR = 0.3, 95% CI 0.2-0.6) and hybrid striped bass (OR = 0.1, 95% CI 0.04-0.3) compared to rainbow trout (Figure 9). The model had acceptable predictive discrimination (ROC = 0.72). The GOF test failed to reject the null hypothesis that the model fit the data (P = 1.000).

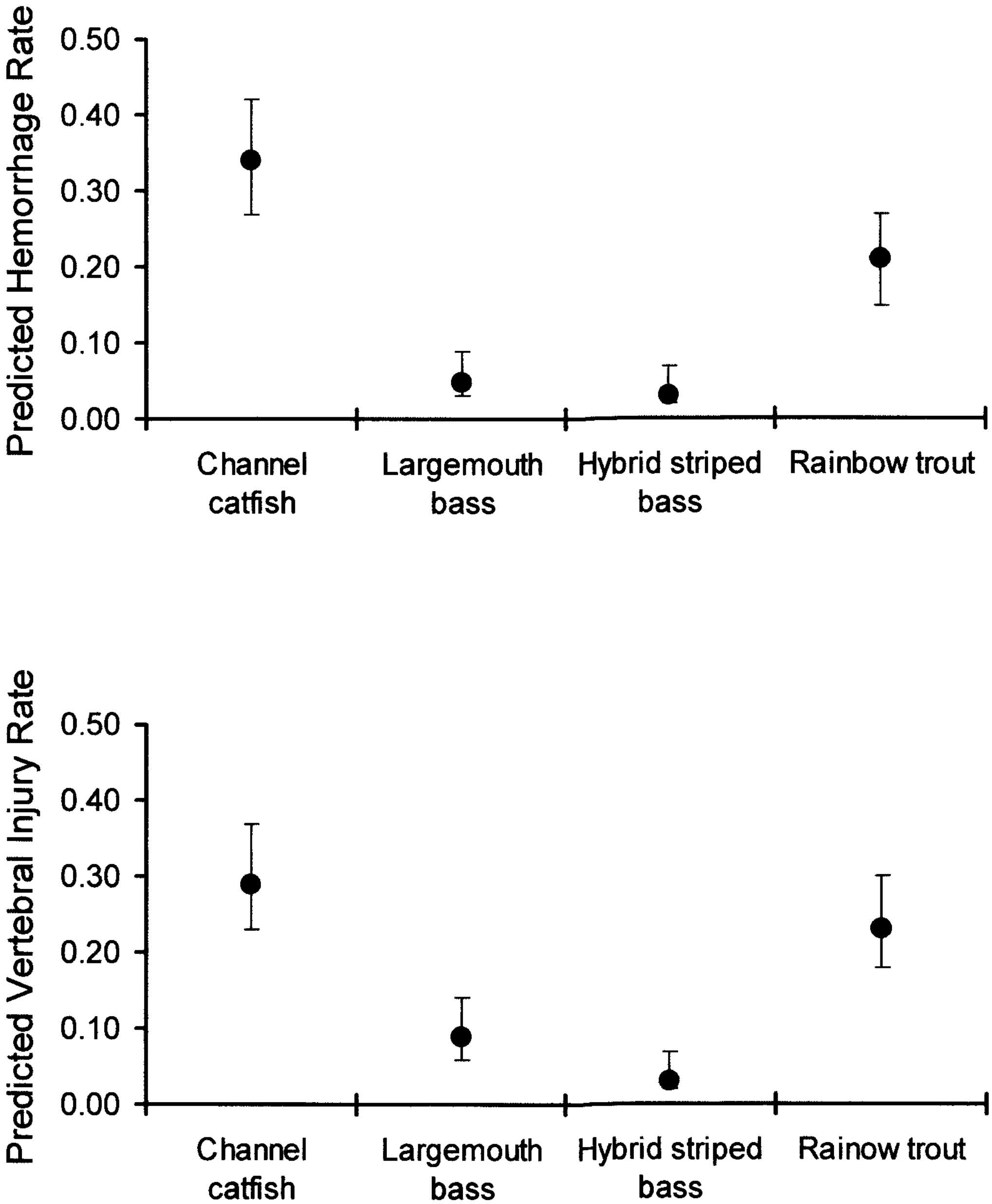


Figure 9.—Predicted injury rates. Model predicted hemorrhage and vertebral injury rate for large (> 230 mm) fish.

Mechanism of Injury.— Evaluation of the pooled data set demonstrated that fish size group (S), fish length (L), evoked fish response (R), and fish species (Sp) were statistically significant single predictors of hemorrhages and vertebral injury. The mechanism of electroshock-induced fish injury can be conceptualized as electrical stimulus, the force of muscle contractions, and resistance to failure of the backbone and associated soft tissues. Lamarque (1967) postulated that stimulation of muscles on both sides of the body simultaneously is the mechanism leading to fracture or dislocation of vertebrae in fish exposed to electrical stimulus. Fish injury is a mechanical phenomenon that occurs when the stresses placed on individual body parts overcome the physical capabilities of the system. In this case, the mechanical properties of the backbone or associated soft tissues are exceeded, resulting in mechanical failure (i.e., fracture or soft tissue injury). The stress placed on the backbone is the force of muscle contraction. The resistance to stress by the backbone is determined by the mechanical properties of the vertebrae and associated soft tissues. Electrical stimulus, the force of contraction, and the resistance to contraction offer a conceptual foundation for the mechanism of electroshock induced injury.

Electrical stimulus.—Surrogate: Fish response (R). Fish behaviors (e.g., forced swimming, immobilization) in electrified water indicate an interruption of normal neuro-motor functioning regardless of whether the interruption is caused by effects on the central nervous system, a stimulus-response type of reaction, or by the direct action of electric current on nerves and muscle. Although there are infinite electrical waveform and field intensity combinations, electrical stimulus (W, E) results in a fish response (R) that is observable. This observable response, which is dependent upon *in vivo* electrical energy, rather than applied electrical energy, was a consistent predictor of injury in the series of experiments (Table 22). Fish response was used as an ordinal variable in the models. The escape response was used as a baseline in the models.

Force of Contraction.—Surrogate: Body size (L). Fish size is strongly correlated with muscle force. Injury is a result of force generated by the simultaneous contraction of parallel myomeres. The total power generated by a fish is proportional to its muscle volume (Videler 1993). Webb and Johnsrude (1988) found maximum power output scaled at a similar rate to muscle mass. Mean myotomal area, total and myotomal weight and myotomal length have been shown to have a strong dependence on fish total length (Archer et al. 1990). When making comparisons of size of animals of different shape, mass has the advantage that almost all animals have a density close to 1.0, making mass a good measure of total volume, a good simplification. However, length is a good measure of similarly shaped animals (Schmidt-Nelson 1977). Fish length is an easily measured surrogate for muscle mass and the forces exerted on the spine during electrical stimulation. Fish length (mm) was used as a continuous variable in the models.

Resistance to Injury.— Surrogate: Vertebral count (V). The resistance to contractual forces is primarily determined by the mechanical properties of the backbone and associated structures. Hamilton et al. (1981) found, in a study evaluating the mechanical properties of brook trout, channel catfish, and bluegill vertebrae, that mechanical properties of vertebrae changed with age and differed among the species. Larger fish, based on within-species comparisons, are more susceptible to injury. There is a strong correlation between fish size (therefore, mass, length, and muscle power) with age. Changes in fish vertebrae structural integrity accompanying maturation (i.e., decrease in vertebral strength) coupled with increased muscle mass and power may account for the increased likelihood of injury in larger fish. Further, the progressive emphasis on caudal locomotion in teleostean evolution is also associated with a trend of decreasing vertebra numbers. Undulating eels may have hundreds of vertebrae, whereas relatively stiff bodied, higher teleosts that rely heavily on the caudal fin for propulsion often have 24 vertebrae or fewer (Gosline 1971). Fish of different species can have vastly different vertebrae size and morphology, and there is a strong correlation between vertebrae size and morphology and mechanical strength. Vertebral count is, therefore, introduced as surrogate for the resistance to the contractual forces. Vertebral count was used as an ordinal variable in the model. Vertebral count intervals were: hybrid striped bass (24); bluegill (28-29); largemouth bass (30-32), channel catfish (42-44); rainbow trout (60-66); and, chinook salmon (67-

75; Scott and Crossman 1973). The 67-75 count interval was used as a baseline in the model.

Mechanistic Models for Electroshock-Induced Injury.— The mechanism-based model (R, L, V) indicated that fish response, fish length, and vertebral count had a significant relation to hemorrhage rate ($P < 0.001$). In the model, hemorrhaging was about 9 times (OR = 8.8, 95% CI 5.1-16) more likely to occur in immobilized fish and about 2 times (OR = 2.4, 95% CI 1.2-4.8) more likely in fish showing forced swimming, compared to those exhibiting the escape response. Hemorrhages increased at a rate of 1.009 (95% CI 1.006-1.0012) per mm of length. Hemorrhage rate differed significantly with fish vertebral count. Hemorrhage was significantly less likely to occur in all vertebral count intervals compared to the 67-75 vertebrae interval: 48 times (95% CI 18-143) less likely in the 24 count interval; 31 times (95% CI 9-200) less likely in the28-29 vertebrae interval; 4 times (95% CI 2-8) in the 30-32 count interval; 2 times (95% CI 1.2-4.7) less likely in the 42-44 count interval; and 2.5 times (95% CI 1.2-5) less likely in the 60-66 count interval. The model had very good discriminatory capability, as indicated by an area under the ROC curve of 0.87). The GOF test failed to reject the hypothesis that the model fit the data $P = 0.134$).

Likewise, the (R, L, V) instrument had very good discriminatory ability when applied to vertebral injury data, as indicated by an area under the ROC curve of 0.86. The GOF test failed to reject the hypothesis that the model fit the data ($P = 0.343$). Significant fish behavioral response, fish size, and vertebral count effects were indicated ($P < 0.001$). Risk of vertebral injury was shown to increase 1.009 (95% CI 1.007-1.012) times for each 1 mm increase in fish length. Vertebral injury was about 5 (95% CI 2.8-8.2) times more likely in fish that were immobilized than in those exhibiting escape responses. Fish that showed forced swimming or escape were at similar risk of vertebral injury (OR = 1.5, 95% CI 0.8-2.7). Compared to the 67-75 vertebral count interval, vertebral injury was: 26 times (95% CI 9-83) less likely in the 24 count interval, 35 times (95% CI 7-500) less likely in the 28-29 count interval, 2 times (95% CI 1.1-4.6) in the 30-32 count interval, .05 times (95% CI 0.2-1.2) as likely in the 42-44 count interval, and 0.8 times (95% CI 0.4-1.6) as likely in the 60-66 count interval.

DISCUSSION

To my knowledge, the value of fish response as a predictor of electroshock-induced injury has not been evaluated prior to this work. Nor have other mechanistic models for electroshock-induced injury been advanced in published works.

The models that best described the relationships of electrical waveform (W), voltage gradient (E), and fish size group (S) varied among the fish species evaluated. In most cases, strong multivariable relationships were demonstrated among these variables in relation to fish injury. Electrical waveform, voltage gradient, and fish size (model W, E, S) were identified as risk factors for electroshock-induced hemorrhage and vertebral injury in chinook salmon and channel catfish and for vertebral injury in largemouth bass. Electrical waveform and fish size group were demonstrated as risk factors by model W, S, for both injury types in rainbow trout. Waveform and voltage gradient (model W, E) were risk factors for hemorrhage in largemouth bass. Electrical waveform (model W) was the single best predictor of both injury types in hybrid striped bass. The identification of risk factors offers guidance to biologist working with these particular species. For instance, biologist working with largemouth bass can reduce the risk of injury to these fish by selecting the least injurious waveform (there was no statistically significant difference in hemorrhage rate between DC and 30-Hz PDC), and minimizing voltage gradient.

Examination of single predictors of injury demonstrated that fish size was the most consistent predictor of injury of both types in the fish examined. The parameters describing electrical stimulus, electrical waveform and voltage gradient, were inconsistent with regard to predictive capacity. In comparison, fish response was a superior predictor of injury, as indicated by the area under the ROC curves. My results indicate that biologists can reduce fish injury by concentrating on eliciting forced swimming behaviors, which are important for successful electrofishing, and by avoiding immobilization of fish.

Evaluation of general models of fish injury demonstrated the significance of fish size and fish response as factors for fish injury. Further, risk of injury differs significantly among fish species, which directly addresses the scope of the electrofishing injury problem. As in the individual experiments, fish response was a strong independent predictor of fish injury, regardless of species, providing biologists with an easily observable means of reducing fish injury. Risk of injury increases dramatically with fish size. My results indicate that in some cases, electrofishing may not be appropriate for use, depending on the size of the fish and species. For instance, large channel catfish and large rainbow trout appear to be very susceptible to injury, regardless of electrical stimulus.

The difference in injury susceptibility among fish species offered a means of exploration of the mechanism of electroshock-induced injury. Simultaneous bilateral contraction of parallel mytomes is hypothesized as the origin of electroshock-induced injury (Lamarque 1990). Thus, injury results from electrical stimulus and the resultant muscle contraction. My experiments demonstrated that injury susceptibility varies among fish species, thereby demonstrating that some fishes backbones are more capable of withstanding the mechanical loads caused by muscle contractions. Fishes designed to withstand the compressive forces caused by caudal locomotion were hypothesized to be more resistant to electroshock-induced injury compared to those fishes designed for undulatory swimming. Lower vertebral counts indicate an evolution toward caudal locomotion and resistance to the compressive forces along the long axis of the vertebral column. Higher vertebral counts indicate an undulatory swimming mode, with smaller vertebrae, and more flexible backbones. Vertebral count is, therefore, an indicator of resistance to compressive forces and the resistance to electrofishing injury.

A statistically significant trend was demonstrated for injury rate to increase with vertebral count. The model incorporating fish response (stimulus), fish size (surrogate for force of contraction), and vertebral count (surrogate for resistance) to contraction offered a conceptual explanation. The mechanistic model offers biologist guidance for prevention and reduction of electrofishing-induced injury. By limiting electrical stimulus to levels below immobilization, risk for fish injury is greatly reduced. Because contractual forces increase with larger fish, care must be exercised when using electricity to capture these fish, especially if the fish of interest has relatively high vertebral counts.

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